DRUGS MODIFYING THE ACTION OF 5-HYDROXYTRYPTAMINE

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FOREWORD

Conditions attributed to the effects of 5-hydroxytryptamine are seen in patients suffering from certain psychological disorders and in patients with carcinoid tumours. In the former there may be too little or too much 5-hydroxytryptamine present in the brain and in the latter, large amounts of 5-hydroxytryptamine are produced by such tumours. There is, therefore, the possibility that substances which act like 5-hydroxytryptamine and substances which antagonise 5-hydroxytryptamine might be of therapeutic use. The study of drugs which modify the action of 5-hydroxytryptamine is, in consequence, of practical as well as academic interest.

SECTION I

INTRODUCTION

INTRODUCTION

The increase in the vasoconstrictor power of blood after clotting has been known for about a hundred years. In 1918, Janeway, Richardson and Park showed that the vasoconstrictor element was contained in an extract of the platelets to a much greater extent than in the rest of the blood. In 1948, Rapport, Green and Page (1948a) isolated the substance from blood serum. It caused constriction of isolated perfused blood vessels and contraction of isolated intestinal strips. In 1949, Rapport finally identified the structure of this vasoconstrictor element as 5-hydroxytryptamine. It was synthesized by Hamlin and Fischer in 1951.

A second group of workers, Vialli and Erspamer (1933), extracted a substance from rabbit gastric mucosa and called it enteramine. This substance caused contraction of isolated smooth muscle and gave a colour reaction after coupling with the diazonium salt of p-nitroaniline. Vialli and Erspamer (1933) thought that it originated from the argentophil cells of the gastro intestinal tract of mammals. In 1952, Erspamer and Asero identified this colour-producing and pharmacologically active substance

(enteramine) as 5-hydroxytryptamine.

Tests for 5-hydroxytryptamine

(i) Isolated preparations:-

The carotid artery ring was the first tissue to be used for the assay of serum vasoconstrictor element (Janeway and Park, 1912). Woolley and Shaw (1952) reported that contractions of sheep carotid artery rings were caused by 0.08 - 0.2 µg./ml. of 5-hydroxytryptamine.

The rat uterus in oestrous was described by Erspamer (1940), who used it for the assay of enteramine. The technique was simplified by Amin, Crawford and Gaddum (1954), who used virgin rats and induced oestrous by injecting stilboestrol a day before the rat was killed. This tissue responded to 1 to 10 ng./ml. of 5-hydroxytryptamine. When atropine was added to the de Jalon solution, no other drug had much activity in this low concentration.

The perfused vessels of rabbit's ear was described by Page (1942), who used this preparation as a test in the isolation of 5-hydroxytryptamine. It was more sensitive than the carotid artery rings.

The rat's colon was described by Dalgleish, Toh and Work (1953). Tests on this preparation, like those on the rat uterus, are usually made in

the presence of atropine. This tissue was more sensitive than the rat uterus in oestrous.

The Venus mercenaria heart was described by Twarog and Page (1953). It responded to between 2 and 20 ng./ml. of 5-hydroxytryptamine.

The superfused isolated anterior byssus retractor muscle of Mytilus edullis was used by Cambridge and Holgate (1955). 5-Hydroxytryptamine caused a rapid relaxation of the electrically stimulated muscle and the amount present in serum diluted 1-10 times could be detected by this tissue easily.

The superfused guinea pig ileum (in the presence of atropine and mepyramine) was described by Cambridge and Holgate (1955) but its sensitivity to 5-hydroxytryptamine was not very great; the smallest amount detectable was 20 ng./ml. in the superfusion fluid.

The isolated hearts of molluscs have been studied by Gaddum and Paasonen (1955). The heart of Spisula Solida, in particular, was quite sensitive to the effects of 5-hydroxytryptamine; it responded to between 0.1 and 1.0 ng./ml.

The rat fundus strip was described by Vane (1957) who found it 10-100 times more sensitive to the action of 5-hydroxytryptamine than the rat uterus in oestrous. The rat fundus strip is the most sensitive preparation for the assay of 5-hydroxy-

tryptamine so far described.

(ii) Chemical methods

Physico-chemical methods are now being introduced to isolate and identify 5-hydroxytryptamine. These methods often give quick and reliable results. Chromatography has played an important part in the extraction of 5-hydroxytryptamine from the tissues. Dalgleish (1956) and Jepson (1955) have summarised with the help of maps, the spots produced by substances related to tryptophan metabolism.

Shepherd, West and Erspamer (1953) used the production of fluorescent derivatives as a simple means of detecting 5-hydroxytryptamine on paper chromatograms. Jepson and Stevens (1953) modified this method to increase its sensitivity. Blaschko and Hellman (1953) reported a colour-reaction (brown) when 5-hydroxytryptamine or tryptamine was incubated with tissues containing amine oxidase. The quantitative estimation of the amounts present depends upon such properties as the production of fluorescent derivatives of 5-hydroxytryptamine. The spectrophotofluorometric method of Udenfriend, Weissbach and Clark (1955) can detect 5-hydroxytryptamine in amounts as small as 50 ng.

General Pharmacological Effects

(i) On the cardiovascular system:

Blood pressure. Page and McCubbin (1953) gave the name "amphibaric" to the type of response which 5-hydroxytryptamine produces in animals, because sometimes it caused a rise and sometimes, even in the same animal, a fall.

In dogs, the blood pressure usually falls with slowing of the heart rate. This depression is followed by a rise and then finally by a prolonged fall. Page and McCubbin (1953) considered the initial fall as a Von Bezold reflex which could be eliminated by cutting the vagi or by giving atropine. In cats, there is a sharp and rather prolonged fall in pressure with slowing of the heart rate. The former effect is partially abolished by atropine or cutting the vagi.

In rats, the response resembles that in cats. A transient fall in pressure is followed by a small rise and this is succeeded by a marked and prolonged fall (Salmoiraghi, Page and McCubbin, 1956). After treatment with ganglion blocking drugs or when the animal is pithed, 5-hydroxytryptamine causes a large rise in pressure.

Page and McCubbin (1953) concluded that the

depressor response to 5-hydroxytryptamine may be due to either the release of endogenous histamine by 5-hydroxytryptamine or/to antagonism of the transmitted peripheral vasoconstriction.

Hollander, Michelson and Wilkins (1957) injected 0.25 to 2 mg. 5-hydroxytryptamine into normal and into hypertensive human beings. They noted an increase in the pulse rate but a variable pressor, depressor or biphasic effect on arterial pressure. An increase in the pulmonary ventilation was consistently present.

Cardiac output. MacCannon and Horvath (1954) reported that by injecting 5-hydroxytryptamine into dogs, an increase in both pulmonary and systemic vascular resistance was obtained. It was quickly followed by a rise in the cardiac output.

A great increase in cardiac output has been shown in human beings, by using Fick's principle (Page, 1957).

Heart muscle and coronary circulation: Schofield and Walker (1953) found that 5-hydroxytryptamine and noradrenaline were equal in potency to adrenaline in causing vasodilation of the perfused anterior descending branch of the left coronary artery of dogs. Bulle (1957) produced cardiac infarct by simply injecting 5-hydroxytryptamine or serum into the left ventricular wall of a perfused rabbit heart. (Chlorpromazine prevented this, but reserpine did not).

Effect of 5-hydroxytryptamine on special vascular areas: Haddy, Fleishman and Emanuel (1957) have shown that adrenaline and noradrenaline caused constriction of the dog's fore-leg small vessels while 5-hydroxytryptamine caused their dilatation. 5-Hydroxytryptamine also caused constriction of the large arteries and veins. Maglini et al (1956) found that 5-hydroxytryptamine caused a local rise in venous pressure in man. Page (1954) reported that 5-hydroxytryptamine is roughly one-tenth as active as adrenaline on the rabbit ear vessels. Rondel et al (1957) found that the response of the blood vessels of the rabbit's ear to 5-hydroxytryptamine was not given by pitressin or acetylcholine.

Roddie et al (1955) injected 5-hydroxytryptamine into the brachial artery of normal human beings and found that it produced a decrease in the blood-flow in the fore arm and hand with marked reddening of the skin and blueness in the fingers. They supposed that 5-hydroxytryptamine caused constriction of the vessels concerned with peripheral resistance, but dilates the minute vessels of the skin.

Effect on pulmonary circulation: Ginzel and Kottegoda (1953) reported that 5-hydroxytrptamine caused vasoconstriction in dogs' and cats' lungs and was more active than adrenaline and noradrenaline. Rudolph and Paul (1957) showed conclusively,

that in dogs 5-hydroxytryptamine was a direct vasoconstrictor of the pulmonary vessels.

Baldrighi and Ferrari (1955) injected 5-hydroxytryptamine intravenously into human beings and showed an increase in venous right atrial and pulmonary arterial pressures. The heart rate and stroke volume was also increased in most cases. Smith and Smith (1955) injected, intravenously into animals, clots formed *in vitro* and noted more profound circulatory effects than with an ordinary suspension of starch granules. 5-Hydroxytryptamine antagonists diminished the effect of the injection of clots and they concluded that 5-hydroxytryptamine was involved in the vascular effects of pulmonary embolism.

McCubbin *et al* (1956) have tried to clarify the chemoreceptor stimulant effect of 5-hydroxytryptamine and have shown that small doses of 5-hydroxytryptamine failed to stimulate respiration unless the drug reaches the chemoreceptors, which are present somewhere in the path of the circulation between the large veins and the left atrium or the ascending aorta. Much larger doses, after section of the sinus nerves, caused the reappearance of a respiratory response; the mechanism of this last mentioned phenomenon, seemed to be central. It persisted after cervical section of the spinal cord

and section of the vagus aortic and sinus nerves.

(ii) On the respiration: This varies from one species to another. Reid and Rand (1952a and b) and Reid (1952) found that when 5-hydroxytryptamine was injected into the right atrium of an anaesthetised dog with intact vagi, there was a brief period of 30 sec. of apnoea followed by tachypnea and simultaneous vasoconstriction of the bronchi.

Schneider and Yonkman (1953 and 1954); Mott and Paintal (1953) and Comroe et al (1953) suggested from their experiments that 5-hydroxytryptamine acts on the receptors situated in the circulation between the large veins and the left atrium or the ascending aorta. When these receptors are stimulated by 5-hydroxytryptamine, the afferent vagal fibres which originate from them cause reflex apnoea. Comroe et al (1953) considered that these 5-hydroxytryptamine receptors were different from those affected by Veratridine, because they could block the 5-hydroxytryptamine receptors with 5-hydroxytryptamine antagonists. Ginzel and Kottegoda (1954) obtained results which showed that excitation of these chemoreceptors (in the aortic and carotid sinus) cause excitation of respiration and that the resulting apnoea is of central origin.

In dogs, the situation is different. Douglas and Toh (1952, 1953); Heymans and Heymans (1953);

Page (1952) and Schneider and Yonkman (1954) found that in normal dogs, the injection of 5-hydroxytryptamine regularly produced a transitory stimulation of respiration followed in half the animals by a period of apnoea. The mechanism of this action is still unsettled but the last mentioned authors think that the initial respiratory stimulation is primarily due to a cardiopulmonary reflex.

Herxheimer (1953) has shown the direct bronchoconstrictor effect of 5-hydroxytryptamine in guinea pigs by making them inhale a 1% aerosol of 5-hydroxytryptamine. The bronchospasm produced in this way is antagonised by lysergic acid diethylamide and atropine but lysergic acid diethylamide has no protective action against anaphylactic shock (Herxheimer, 1955).

(iii) Other effects on the kidneys: Erspamer and his associates have investigated the effects of 5-hydroxytryptamine on the kidney in detail (Erspamer and Ottoleughi, 1950-1953). Erspamer (1954) concluded that the antidiuretic effect of 5-hydroxytryptamine was due to a preferential vasoconstriction of the different glomerular arterioles. He considered 5-hydroxytryptamine as a hormone designed for the physiological regulation of renal function.

Pickford (1957) reported on her work done with

Abraham (1956) in conscious dogs. There was an antidiuretic effect with 5-hydroxytryptamine only when its injection raised the blood-pressure and thus reduced the glomerular filtration rate and renal plasma flow. This suggested that these responses were of reflex origin and that the blood vessels of the kidney show no particular sensitivity to 5-hydroxytryptamine.

(iv) On the central nervous system: Whether 5-hydroxytryptamine has any function in the central nervous system or not is still in dispute, but experimental evidence strongly suggests that it has. In 1943, Hofmann discovered that small quantities of lysergic acid diethylamide (inhaled by accident in the laboratory) caused hallucinations and a peculiar state apparently similar to drunkenness in man. Gaddum (1953b) showed that lysergic acid diethylamide antagonised the effects of 5-hydroxytryptamine on the isolated rat's uterus preparation and suggested that there might be a connection between this property and the ability to cause hallucinations. 5-Hydroxytryptamine had been shown to be present in the brain by Amin, Crawford and Gaddum (1954) and independently by Twarog and Page (1953). Gaddum (1953a) suggested that hallucinations caused by lysergic acid diethylamide might arise from its antagonism of the effects of 5-hydroxytryptamine in

the brain. Woolley and Shaw (1954a), independently, suggested that substances which antagonised 5-hydroxytryptamine caused "mental aberrations".

Since then, substances have been found such as ergometrine, dibenamine and bromolysergic acid ethylamide which antagonise 5-hydroxytryptamine on isolated tissues but which do not produce effects on the central nervous system. In addition, substances, such as mescaline, have been found to produce effects on the brain somewhat similar to those of lysergic acid diethylamide but not to antagonise the effects of 5-hydroxytryptamine on the rat uterus (Gaddum, 1957b). These findings are not what would be expected if there is a connection between the ability to antagonise 5-hydroxytryptamine but as Gaddum (1957b) remarks, "They do not disprove the theory.....but they do diminish the value of the existing evidence favouring the theory".

N.B. Costa (1956) reported that a concentration of lysergic acid diethylamide lower than 1 ng. per ml. caused facilitation of the responses of 5-hydroxytryptamine on the rat uterus, in addition to the antagonistic action at higher concentration, and that this effect was also shown by mescaline in a concentration of 100 ng. per ml. He suggested that this facilitation was the typical effect of drugs which caused hallucination. He also observed antagonism between 5-hydroxytryptamine and certain tranquillisers like reserpine, chlorpromazine and azacyclonal. Cerletti and Doepfner (1958) failed to obtain such a facilitation with lysergic acid diethylamide on the rat uterus and I also, have not been able to repeat Costa's results with lysergic acid diethylamide.

Feldberg and Sherwood (1954) reported the effects of 5-hydroxytryptamine introduced into the cerebral ventricles of conscious cats. Doses of 75-500 µg. 5-hydroxytryptamine produced loss of muscular tone, tachypnea, licking movements, bursts of profuse salivation, tremors of head and twitching of eyelids. Gaddum and Vogt (1956) repeated this work and found, further, that the depressant action of 5-hydroxytryptamine in the brain of cats was antagonised by lysergic acid diethylamide, ergometrine, morphine, methadone and amphetamine. Bromolysergic acid diethylamide, 5-hydroxytryptamine and methyl medmain were without such an effect. They concluded that this type of antagonism was probably not related to the antagonism of 5-hydroxytryptamine by lysergic acid diethylamide on peripheral tissues. (See section on antagonists on page 30).

Vogt (1957) reported the interaction of 5-hydroxytryptamine and lysergic acid diethylamide in the brain in experiments where multiple electrodes were introduced into the brain of cats. Records were made of the e.e.g. changes when 5-hydroxytryptamine was injected into the lateral ventricles, either alone, or in combination with lysergic acid diethylamide (Vogt, Gunn and Sawyer, 1957). A reduction in the amplitude of electrical activity was

noted in most of the areas. In these experiments, 5-hydroxytryptamine and lysergic acid diethylamide each caused a characteristic pattern of electrical changes, which coincided in time with the behavioural changes observed in conscious cats. When the drugs were given together, the effects were additive at some sites and antagonistic at others. These findings favour the theory that antagonistic effects of 5-hydroxytryptamine and lysergic acid diethylamide on behaviour depends on selective sensitization or inhibition of a characteristic group of centres by each drug and not on simple interaction by competition for the same receptors within the brain.

Shore, Silver and Brodie (1955a and b) and Brodie et al (1955) observed that 5-hydroxytryptamine and reserpine both markedly increased the hypnotic action of hexobarbitone in mice and that both these effects are antagonised by the prior administration of lysergic acid diethylamide. After reserpine, the 5-hydroxytryptamine content of certain tissues, such as the brain and platelets is lowered and it has been suggested that the tranquillising effect of reserpine may be due to the liberation of 5-hydroxytryptamine in the brain.

Gaddum and Giarman (1956) have shown the presence of 5-hydroxytryptophan decarboxylase, which forms 5-hydroxytryptamine, in different tissues,

including some parts of the brain. Bogdanski et al (1957) have shown an apparent relationship between 5-hydroxytryptamine and the decarboxylase activity in the brain, suggesting that 5-hydroxytryptamine is synthesised in the brain. Benitz, Murray and Woolley (1955a and b) reported that ~~the~~ rat and human oligodendroglia cells in tissue cultures were thrown into strong contractions by the addition of 5-hydroxytryptamine to the nutrient fluid. Woolley and Shaw (1957a) suggest that this phenomenon is the cerebral counterpart of the effects of 5-hydroxytryptamine on the smooth muscles.

Gieger (1957) described a pumping movement in human and rabbit neurones grown in tissue cultures which is initiated by 5-hydroxytryptamine in concentration of 0.5 to 2 µg. per ml.

Yet another aspect of the action of 5-hydroxytryptamine in the brain was shown by Marrazzi and Hart (1955) who found that 5-hydroxytryptamine, like adrenaline and noradrenaline, blocked synaptic transmission in the brain. It was actually the most active of these inhibitors. Gluckman, Hart and Marrazzi (1957) showed that the cerebral synaptic inhibitory activity of 5-hydroxytryptamine was 20 times that of adrenaline but, on the ciliary ganglion 5-hydroxytryptamine was much less active.

Bradley (1957) studied the effects of 5-hydroxytryptamine on the behaviour of conscious cats and the electrical activity of the brain. Small doses of lysergic acid diethylamide, which only "alerted" the animal did not antagonise the effects of 5-hydroxytryptamine but larger doses of lysergic acid diethylamide, such as used by Gaddum and Vogt (1956), did do so. The combination of 5-hydroxytryptamine and lysergic acid diethylamide together produced a new pattern of electrical activity, different from those of either drug alone. Bradley considered that 5-hydroxytryptamine affected the centres influencing the e.e.g. less than other areas of the central nervous system.

(v) On the adrenal medulla: Reid and Rand (1952a,b) found that 5-hydroxytryptamine, when injected into the cut stump of the superior mesenteric artery of the eviscerated cat, caused a discharge of adrenaline from the adrenal medulla. There was a rise in blood pressure, increase in heart rate, dilatation of the pupils and contraction of the nictitating membrane. Reid (1952) also showed that tryptamine did not do this, whereas bufotenine and bufotenidine (Raymond Hamet, 1942-1943) did it to a greater extent even than 5-hydroxytryptamine.

(vi) Histamine release: Feldberg and Smith (1953) showed that 5-hydroxytryptamine and trypta-

mine released histamine in perfused isolated skin flaps and gastrocnemius muscles of cats and dogs, as well as on rat tissues. Their activity was only 1/100 times that of compound 48/80. This does not exclude the possibility that histamine release is at the root of certain phenomena, resembling the effects of histamine, which are seen in intact animals after they have been injected with the tryptamine-like compounds.

(vii) On coagulation of the blood: Correll et al (1952) studied the haemostatic effect of 5-hydroxytryptamine in the blood of rats, guinea pigs, chickens and rabbits and showed that it had marked activity, but that the effect was short lived. Page (1948) also suggested such an effect. Shore et al (1956) reported that reserpine treatment, which resulted in the loss of 90% of platelet 5-hydroxy-tryptamine, did not produce any significant effect on the bleeding time in rabbits, rats and guinea pigs. So the participation of 5-hydroxytryptamine in the process of blood coagulation is still not clear.

(viii) On the capillary permeability: Rowley and Benditt (1956) have shown in rats (which had already received injections of Vital dye) that a subcutaneous injection of 5-hydroxytryptamine pro-

duced a marked increase in capillary permeability. This latter effect was shown by the local exudation of the Vital dye at the site of injection of the 5-hydroxytryptamine. They have also shown that 5-hydroxytryptamine was 200 times as effective as histamine in producing oedema in the rats' paw. Sparrow and Wilhelm (1957) found that this effect of 5-hydroxytryptamine varied greatly from one species to another. It was very marked in rats but not in guinea pigs and rabbits.

(xi) On the gastro-intestinal tract: Long before the true nature of 5-hydroxytryptamine was identified, Erspamer and Vialli (1933) had shown that enteramine was present in the rabbit's gastric mucosa. Since then, its distribution in the gastro-intestinal tract in various animals and human beings has been thoroughly investigated by Erspamer and others (work reviewed by Erspamer,(1954); Feldberg and Toh (1953) and Dalgleish, Toh and Work (1953). It is distributed widely in the mucosa of the gastro intestinal tract of the different species.

Erspamer (1954) states that 5-hydroxytryptamine is formed in the enterochromaffin cells of the gastro intestinal mucosa and is released into the circulation where it is taken up by the platelets. 5-Hydroxytryptamine stimulates the smooth muscles

of some parts of the gastro intestinal tract in certain species (Erspamer, 1954; Vane, 1957). The sensitivity of the different tissues varies greatly. The guinea pig ileum is extensively used by various workers in the 5-hydroxytryptamine field. Gaddum (1953a) postulated that 5-hydroxytryptamine and tryptamine cause contraction of this tissue by acting on the same set of receptors. A large dose of 5-hydroxytryptamine desensitised the preparation to 5-hydroxytryptamine and tryptamine, but substance P was still active. On the other hand, a large dose of substance P left the tissue insensitive to the action of substance P but it responded to 5-hydroxytryptamine and tryptamine. Rocha e Silva, Valle and Picarelli (1953) concluded that the action of 5-hydroxytryptamine on the guinea pig ileum was cholinergic in origin because it was blocked by atropine. This cholinergic effect was not blocked by hexamethonium, so presumably it was not at the ganglion. The provisional conclusion drawn was that 5-hydroxytryptamine acted on the post ganglionic fibres of the intra nervous system, more peripherally than where nicotine acts.

Gaddum and Hameed (1954) failed to get total suppression of 5-hydroxytryptamine responses by lysergic acid diethylamide on the guinea-pig ileum.

Increasing the concentration of lysergic acid diethylamide 100 times did not increase the antagonism to 5-hydroxytryptamine. They suggested that 5-hydroxytryptamine receptors in the guinea pig ileum were different from the 5-hydroxytryptamine receptors in the rat uterus and the rabbit ear vessels, because of their resistance to the action of lysergic acid diethylamide and their easy paralysis by excess of 5-hydroxytryptamine itself.

Cambridge and Holgate (1955) made similar observations with atropine. They plotted the responses to a constant dose of 5-hydroxytryptamine, histamine and acetylcholine against the logarithm of the dose of atropine. The curves obtained with 5-hydroxytryptamine had a plateau over the range of concentration 0.01 to 1.0 µg./ml. of atropine, while the curves obtained with histamine and acetylcholine were both simply S-shaped.

Gaddum and Picarelli (1957) classified the actions of 5-hydroxytryptamine on the tryptamine receptors in the guinea pig ileum as being of two types: a) at "M" receptors which were blocked by morphine and b) at "D" receptors which were blocked by dibenzyline. Atropine, cocaine and methadone blocked the effect of 5-hydroxytryptamine on the "M" receptors, i.e. after the action of dibenzyline,

but had no additional effect after morphine. Lysergic acid diethylamide, dihydroergotamine and 5-benzyloxygramine blocked the effects of 5-hydroxytryptamine on the "D" receptors, i.e. after morphine, but were without any effect after dibenzyline. The morphine sensitive receptors were probably in the nervous tissue and the dibenzyline sensitive receptors were probably in the muscle.

Gaddum (1957a) compared the mode of action of 5-hydroxytryptamine with that of acetylcholine. Both drugs act on two different types of receptors which are blocked by two separate groups of antagonists. The ganglionic effect of acetylcholine was compared with the action of 5-hydroxytryptamine on the "M" type of receptors in the guinea-pig ileum. The former was specifically blocked by hexamethonium and the latter by morphine, less specifically, because nicotine is also blocked to some extent. Similarly, atropine blocked the effects of acetylcholine on the smooth muscle while lysergic acid diethylamide blocked the effect of 5-hydroxytryptamine on the plain muscle of the guinea pig ileum.

Kosterlitz and Robinson (1955) showed that the contraction of the guinea pig ileum caused by the application of 5-hydroxytryptamine to the bath was

different from the one obtained by filling the lumen of this tissue. The latter was not blocked by dibenamine, cocaine or by large doses of 5-hydroxytryptamine. Both types of contractions were partially inhibited by morphine. Kosterlitz and Robinson (1957) also showed that small amounts of 5-hydroxytryptamine added to the outside of the intestine depressed or even abolished the peristaltic reflex. Bülbring and Lin (1957) confirmed that 5-hydroxytryptamine added outside the intestine blocked peristalsis but when added inside the lumen of the guinea pig ileum, it caused an increase of peristaltic movement and a lowering of the threshold pressure. The contractions obtained were more frequent and a larger volume of gluid was propelled. Bülbring and Lin (1957) suggested that 5-hydroxytryptamine acted on the sensory receptors in the mucosa, which trigger the peristaltic reflex, and that the formation of 5-hydroxytryptamine by intestinal mucosa may be part of the mechanism required for peristalsis.

According to Page (1954) it has been known for a very long time that 5-hydroxytryptamine causes evacuation of the bowels even in animals under deep anaesthesia. This effect was noted in experimental animals by Erspamer (1952) and confirmed by Collier (1957). A dose of 5 mg./kg. of

5-hydroxytryptamine injected into mice caused an increase in the number of faeces passed within four hours of treatment. Some mice passed unformed faeces, "as much as with a large dose of senna". These facts, together with the presence of diarrhoea in patients with carcinoid tumours, prompts the question - "Is 5-hydroxytryptamine nature's remedy for constipation?" (Collier, 1957).

Black, Fisher and Smith (1958) found that 5-hydroxytryptamine, when intravenously infused in a dog for 45-90 mins. did not evoke acid gastric secretion but only the secretion of mucous. Histamine evoked acid gastric secretion was inhibited by a concurrent infusion of 5-hydroxytryptamine for 30 mins. but giving 5-hydroxytryptamine at the start of such a histamine infusion did not prevent the response when both the vagi in the neck were cut. 5-Hydroxytryptamine did not inhibit histamine-stimulated secretion

Distribution of 5-hydroxytryptamine

The distribution of 5-hydroxytryptamine has been reviewed by Erspamer (1954). It is present in all the tissues containing cells belonging to the enterochromaffin system. It has been found in a number of vertebrates, except in Teleostei and Cyclo-

stomata (groups of fish), which do not contain enterochromaffin cells.

Its occurrence in the intestine of a number of species has been reported by Erspamer and Faustini (1953). Dalgleish, Toh and Work (1953) found 4-10 µg./g. in the dog's muscularis-mucosa but none in the muscularis-externa.

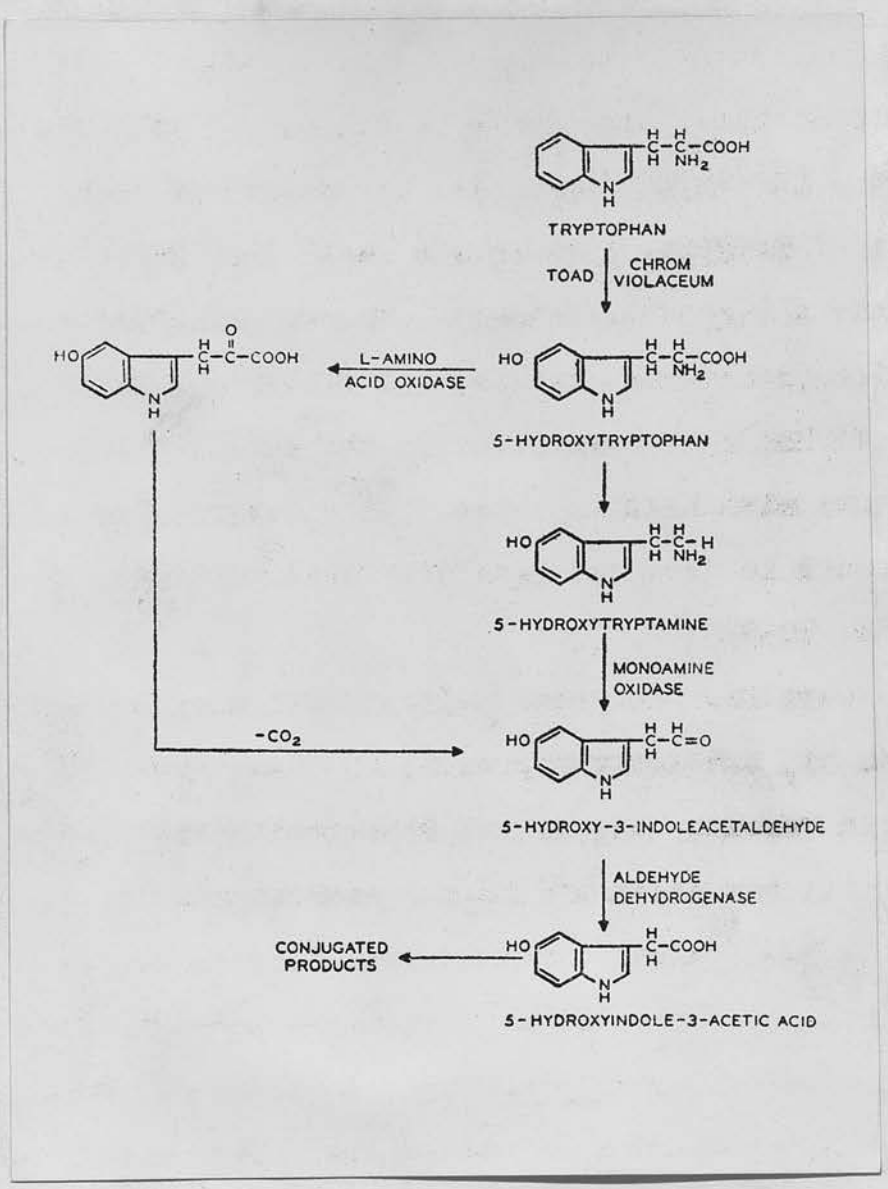
It was shown to occur in the brain by Twarog and Page (1953) and, independently, by Amin, Crawfor and Gaddum (1954). The latter found the distribution resembled that of noradrenaline found by Vogt (1954).

The platelets are very rich in 5-hydroxytryptamine. Humphrey and Toh (1954) found that dog's platelets contain 0.5 - 2.7 µg./10^{-9} platelets but the rest of the blood contains only a very small quantity of 5-hydroxytryptamine.

Garven (1955) found significant quantities in the spleen and serum of rabbits (as well as in the gut mucosa and blood). A smaller amount was present in the hypothalamus, liver and bone-marrow. Skeletal muscle, nerves and adrenals contained no 5-hydroxytryptamine.

The amounts in lung tissue have been studied by Weissbach, Waalkes and Udenfriend (1957), who found very little in guinea pig lung but much more in lungs from mice, rats and rabbits.

Figure I



Various pathways of 5-hydroxytryptamine metabolism (Udenfriend (1957)

In invertebrates, it has been found in ganglia and peripheral nerves of Sepia (Florey and Florey, 1953, 1954; and Florey, 1954), and in the visceral ganglia of Venus mercenaria (Welsh, 1954). It occurs in large quantities (5 mg./g.) in carcinoid tumours (Lembeck, 1953), and is present in mast cells of rats (Benditt et al, 1955) and, in large amounts along with histamine, in mast cell tumours in mice (Sjoerdsma, Waalkes and Weissbach, 1957).

It has also been found in the spinal fluid of patients with head injuries, brain tumours and meningitis and in dogs and cats with head injuries (Sachs, 1957).

Apart from numerous sources listed by Erspamer (1954a,b), 5-hydroxytryptamine has been found in certain venoms. Jaques and Schachter (1954) found it in the tentacles of the sea anemone and in wasp, but not bee, venom. Collier and Chester (1956) found 5-hydroxytryptamine together with an inactivating enzyme, in nettle stings and Adam and Weiss (1956) found as much as 304 μg./mg. (dry weight) in scorpion venom.

Metabolism of 5-hydroxytryptamine

Fig. I shows the way in which 5-hydroxytryptamine may be formed and broken down in nature. Tryptophan is oxidised to 5-hydroxytryptophan in the toad and in chromobacterium violaceum (Mitoma et al,

1955, 1956). 5-Hydroxytryptophan, which can pass the blood brain barrier (Udenfriend et al, 1956, 1957), appears to be an intermediate in the synthesis of 5-hydroxytryptamine. When fed to animals, there is a large rise in the 5-hydroxytryptamine content of the brain and when it is given to human beings there is a large rise in the excretion of 5-hydroxy-indole acetic acid (Davidson et al, 1957). 5-hydroxytryptophan decarboxylase, which would convert 5-hydroxytryptophan into 5-hydroxytryptamine, is widely distributed throughout the body. It occurs in large amounts in the kidney, liver and stomach but not in platelets (Udenfriend and Weissbach, 1954). It is also found in sympathetic ganglia and in the brain (Gaddum and Giarman, 1956), where its distribution is reported to be similar, in dogs and cats, to that of 5-hydroxytryptamine (Bogdanski; Weissbach and Udenfriend, 1957), except in the pyriform cortex and amygdala. The caudate nucleus contains the highest decarboxylase activity.

Tryptamine is known to be destroyed by amine oxidase in vitro (Blaschko, Richter and Schlossmann, 1937; Pugh and Quastel, 1937), and this enzyme might be expected to attack 5-hydroxytryptamine also. Rapport, Green and Page (1948b) found that serotonin was destroyed by lung extracts and Blaschko (1952)

showed that serotonin and enteramine (5-hydroxytryptamine) were inactivated by amine oxidase. This enzyme is widely present in mammals, especially in the brain, but is absent from blood and skeletal muscle (Blaschko, 1957). It is present in the rat fundus strip (Vane, 1959).

This enzyme also destroys mono-methyl tryptamine, in which the methyl group is attached to the side-chain amino group, but will not destroy, and is inhibited by, the analogous dimethyl, diethyl or benzyl compounds (Gover, Howes and Gibbons, 1953).

If 5-hydroxytryptamine is destroyed by amine oxidase, this would explain why Marsilid, an amine oxidase inhibitor, potentiates the effects of 5-hydroxytryptamine (Udenfriend et al, 1957). Sjoerdsma et al (1955) are convinced that oxidative deamination by amine oxidase is the major metabolic pathway of 5-hydroxytryptamine.

The product of oxidation is 5-hydroxyindole acetaldehyde which should be rapidly oxidised to 5-hydroxyindoleacetic acid. Excretion of this compound appears to be the main route of disposal in man but in other species urinary output is small and other pathways must be considered (Udenfriend, 1957). Titus and Udenfriend (1954) reported excretion rates per day of 2-3 mg. 5-hydroxyindoleacetic acid for dogs and 10 mg. for man. It is

excreted in larger quantities by patients with malignant carcinoids but in smaller amounts (1-1.9 mg./day) by patients with collagen vascular disease (Haverback, Sjoerdsma and Terry, 1955).

Carcinoid tumours

In 1952, Biörck, Axen and Thorson reported a 19-year old man suffering from dyspnoea and a curious type of cyanosis. The post mortem examination revealed pulmonary valvular stenosis and an ileal argentaffinoma with secondary deposits in the liver. An addendum was added to the paper describing two more cases. Lembeck (1953) demonstrated the presence of 5-hydroxytryptamine in the carcinoid tumour. It was evident that the signs and symptoms of carcinoid tumours were due to hyper secretion of 5-hydroxytryptamine and Page et al, 1955 reported the excess excretion of 5-hydroxyindoleacetic acid in these patients. The serum 5-hydroxytryptamine content of carcinoid cases has been shown to be high by various workers (Duncan et al, 1955; Pernow and Waldenström, 1957 and Waldenström et al, 1956) and Lembeck and Neubold (1955) have also shown that patients with carcinoid tumours excreted high quantities of 5-hydroxytryptamine in urine. Pernow and Waldenström (1957) have suggested that histamine liberation might also contribute to the symptoms of

carcinoid disease. Udenfriend et al. (1956) reported that 6% of the dietary tryptophan at a daily in take of 500 mg. is converted to urinary 5-hydroxyindoleacetic acid in patients with carcinoid tumour. In normal individuals only 1% of tryptophan is metabolised in this way. Mattingly (1956) reported that actually only 25% of all patients with malignant carcinoids of the small intestine, show clinical symptoms.

Page (1958) has concluded that none of the antagonists so far available have been successful in the treatment of patients with malignant carcinoids. "Often they work in isolated preparations with high specificity, only to be ineffective in the whole organism".

Antagonists of 5-hydroxytryptamine

(i) Lysergic acid derivatives: Hofmann's discovery of the central effects of lysergic acid diethylamide showed that this was an interesting compound, but it was Gaddum (1953b) who showed that this property might be due to its antagonism of the action of 5-hydroxytryptamine and who showed that lysergic acid diethylamide was an antagonist of 5-hydroxytryptamine on the rat uterus. Gaddum and Hameed (1954) found lysergic acid diethylamide to be an active and specific antagonist to the

action of 5-hydroxytryptamine on the rabbit ear but on the guinea pig ileum lysergic acid diethylamide, in a concentration of 100 ng./ml. reduced the 5-hydroxytryptamine responses by 50% only. Concentrations of lysergic acid diethylamide 100 times more did not have any additional effect on 5-hydroxytryptamine responses on the guinea pig ileum. This finding led them to suggest the two sets of 5-hydroxytryptamine receptors in the guinea pig ileum already mentioned (page

2-Bromo-lysergic acid diethylamide (BOL) inhibits the action of 5-hydroxytryptamine on the rat uterus (Sollero, Page and Salmoiraghi, 1956 and Savini (1956) showed that BOL blocks the vasoconstrictor action of 5-hydroxytryptamine on the perfused rabbit ear and on this preparation is 1/10th as active as lysergic acid diethylamide. Unlike lysergic acid diethylamide and ergometrine it never caused vasoconstriction itself. Cerletti and Konzett (1956) have investigated the antagonistic properties of a number of derivatives of lysergic acid diethylamide on the isolated rat's uterus and perfused rat's kidney. Acetyl- and Bromo-lysergic acid diethylamide were the most active compounds. Lysergic acid diethylamide antagonised the anti-diuretic effect of 5-hydroxytryptamine in rats

(Del Grego, Masson and Corcoran, 1956), and partially prevented asthma caused by aerosols of 5-hydroxytryptamine in guinea pigs (Herxheimer, 1955). Another potent compound is 1-methyl-2-bromo-lysergic acid diethylamide (Cerletti and Doepfner, 1958).

Salmoiraghi, McCubbin and Page (1956) found species differences in the ability of lysergic acid diethylamide and bromo-lysergic acid diethylamide to effect the vascular action of 5-hydroxytryptamine. Both the compounds prevented the pressor and depressor responses to 5-hydroxytryptamine over long periods in the rats, but in dogs, much larger doses of bromo-lysergic acid diethylamide caused only transient and irregular blockade. In cats, both these drugs failed to have any effect against the pressor responses to 5-hydroxytryptamine while in the isolated vascular beds of cats, both the drugs were more effective to block the action of 5-hydroxytryptamine.

Ergotamine was shown to block the vasoconstrictor effect of defibrinated blood by Heymans, Bouckaert and Moraes (1932) and Page and McCubbin (1953) reported that this drug reversed the pressor action of 5-hydroxytryptamine while increasing the pressor action of some other drugs. Gaddum (1953c) found dihydroergotamine to be an active antagonist

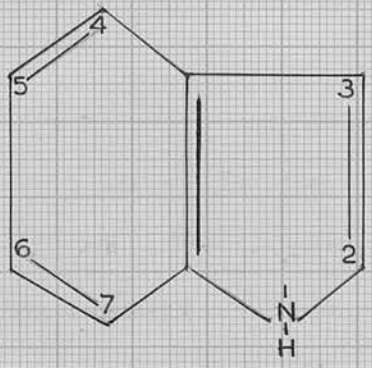
of 5-hydroxytryptamine on the isolated rabbit ear preparation. Shaw and Woolley (1953) found that yohimbine, ergotamine and ergotoxine antagonised the effects of 5-hydroxytryptamine on the carotid artery rings. Savini (1956) reported that ergotamine antagonised the constrictor effect of 5-hydroxytryptamine on the isolated rabbit ear preparation and Page and McCubbin (1953) reported that yohimbine blocks the pressor action of 5-hydroxytryptamine in dogs.

(ii) Adrenergic blocking drugs. Dibenamine has been reported to be a powerful antagonist to 5-hydroxytryptamine in its action on the rat's uterus and in its antidiuretic effect (Erspamer and Correale, 1953; Fingl and Gaddum, 1953). This compound is known to have a prolonged antagonistic effect against adrenaline and some antihistamine effect also (Nickerson, 1949). Gaddum and Hameed (1954) found that it blocked the action of 5-hydroxytryptamine on the rat uterus much more than on the isolated rabbit ear and guinea pig ileum. Furchgott (1954) studied the effect of dibenamine on the contractile responses of strips of rabbit aorta, produced by a variety of stimulating drugs. It was most effective against the sympathomimetic amines and then in order of decreasing effectiveness against 5-hydroxytryptamine, histamine and acetylcholine.

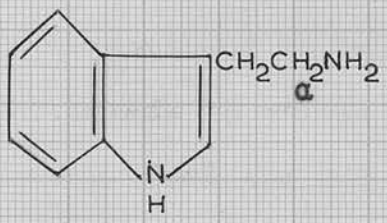
He has shown that by keeping a high concentration of one of the above mentioned stimulant drugs in the bath, during exposure of the strips to dibenamine, he obtained partial or even complete protection against the blocking effect of dibenamine. This he called "self-protection" and suggested that dibenamine exerted its blocking action by reacting in an essentially irreversible manner with the free receptors, with which the stimulant drugs combined and caused contraction.

Dibenzyline, another antiadrenaline drug, has been shown by Gaddum and Picarelli (1957) to block one set of 5-hydroxytryptamine receptors in the guinea pig ileum, more or less permanently. Responses to histamine were also effected with such treatment with dibenzyline but not those to nicotine. Holzbauer and Vogt (1955) found substances which had antagonised the inhibitory action of adrenaline on the rat uterus. All the nine substances, which they studied, also antagonised 5-hydroxytryptamine but did not disturb responses to choline esters. They did not find a substance which blocked 5-hydroxytryptamine without also blocking adrenaline. They pointed out the complete unpredictability of the effects which even chemically related drugs exert on the inhibitory effects of adrenaline on the uterine muscle.

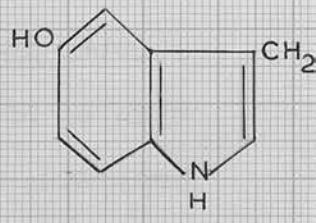
FORMULAE



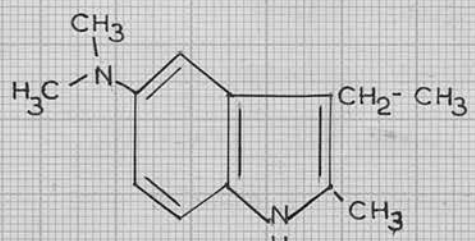
Indole



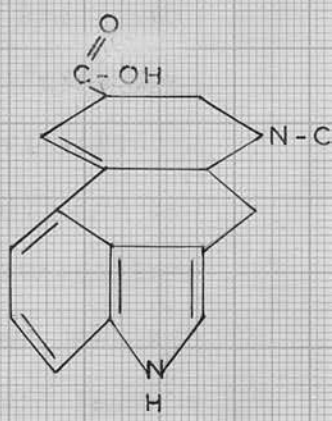
Tryptamine



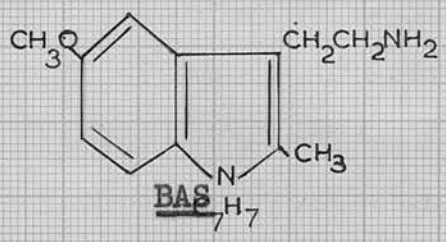
5-Hydroxytryptamin



Medmain



Lysergic acid



BAS$_7H_7$

(iii) Analogues of 5-hydroxytryptamine. The first synthetic analogues of 5-hydroxytryptamine to be tested were prepared by Woolley and Shaw (1952a, 1952b) who referred to these substances as "anti-metabolites". They reported that 2-methyl-3-ethyl-5-amino indole was an antagonist of 5-hydroxytryptamine on pieces of the carotid artery of sheep. This compound, when fed to dogs in 500 mg. daily doses for 5 days, prevented the pressor response to injected 5-hydroxytryptamine but Spies and Stone (1952) and Page and McCubbin (1953a) failed to demonstrate any change in the blood pressure of hypertensive patients with this compound. Iverson and Bul (1953) found 2:3-dimethyl-5-amino indole ineffective in hypertensive cases although this compound was shown by Woolley and Shaw (1953) to prevent the rise in blood pressure in dogs due to 5-hydroxytryptamine even when given by mouth.

Shaw and Woolley (1954) found that substitution of methyl or other alkyl groups to the amine group in 2 methyl-3-ethyl-5-amino indole compounds gave very active antagonists of 5-hydroxytryptamine. Medmain (2-methyl-3-ethyl-5-dimethylamino indole) and its 1 methyl derivative were about 250 times as active as the parent, unalkylated amine (2-methyl-3-ethyl-5-amino indole). Medmain was a very active

antagonist of 5-hydroxytryptamine on the carotid artery and isolated rat uterus. When added to the bath in concentrations higher than that required to block the action of 5-hydroxytryptamine, the analogue itself caused stimulation of the uterus (this was not seen on the carotid artery rings).

The closely related compound, 1-methylmedmain did not have this stimulant property. Contractions produced by medmain on the rat and guinea pig uterus were antagonised by 2-methyl-3-ethyl-5-amino indole. 3-(β-dimethylaminoethyl)-5-amino indole (Shaw and Woolley, 1954) also, like medmain, antagonised the effects of 5-hydroxytryptamine and stimulated the tissue in higher concentration. The stimulant action of medmain usually took the form of repeated contractions of the rat uterus, which persisted even after repeated washings.

Intraperitoneal injections of medmain into mice resulted in convulsions but 1-methylmedmain, which does not have any stimulant action, did not cause any convulsions in mice. Woolley and Shaw suggested that these convulsions might be a 5-hydroxytryptamine-like effect of medmain in the central nervous system.

Page and McCubbin (1953b) found that medmain inhibited the pressor but not the depressor action of 5-hydroxytryptamine in dogs and cats and it

prevented respiratory stimulation as well. However, it had no effect in dogs by mouth and when fed to hypertensive dogs and patients with malignant hypertension did not prove of any value.

Woolley and Shaw (1952a) reported that 2-5-dimethyl serotonin was a potent antagonist of the action of 5-hydroxytryptamine on the isolated rat uterus and on dog blood pressure and that 2-5-dimethyl bufotenine was slightly less potent (Woolley and Shaw, 1956a). Substitution in the 2-position yielded antagonists but substitution in 1-position gave 5-hydroxytryptamine-like substances. 1-benzyl-2,5-dimethyl 5-hydroxytryptamine (BAS) was the most active antagonist, when tested in doses of 1 mg/Kg. in dogs against the pressor action of 5-hydroxytryptamine (Shaw and Woolley, 1956a). Woolley and Shaw (1956) suggested that 5-hydroxytryptamine might be associated with the cause of the essential hypertension and that BAS would be of therapeutic use in such a condition. Wilkins (1956) reported that the beneficial effects of BAS were similar to those of reserpine in 25 hypertensive patients. Wilkins and Hollander (1957) found that BAS alone, or in combination with other drugs, reduced the blood pressure of about 25% of patients suffering from hypertension. They were not sure whether

this effect was due to the antagonism of 5-hydroxy-tryptamine or not.

Quadbeck and Röhn (1954) prepared and studied a series of 31 substituted gramine and tryptamine derivatives. 5-fluoro, 5-chloro, 5-methoxy, 5-methyl and 5-bromo tryptamine were more or less active synergists on isolated guinea pig colon. While 5-fluoro, 5-methyl and 5-chloro tryptophan were antagonists of 5-hydroxytryptamine. There was a marked increase in the antagonistic potency when the dimethylamino group was introduced into the 3-position and an even greater one when introduced into the 5-position. The most active compound was 2-methyl-5-chlorogramine.

Erspamer (1955) tested 9 gramine derivatives against the effect of 5-hydroxytryptamine on the rat uterus, and compared their activity with 3 representative amino indoles of Woolley and Shaw. 2-methyl-5-amino gramine was the most active and 2-methyl-5-bromo gramine was the least. These active gramines, *in vitro*, had negligible effect when tested *in vivo* for antidiuretic activity. Erspamer suggested that these drugs may be rapidly destroyed in the body.

Gaddam, Hameed, Hathway and Stephens (1955) studied a number of synthetic indole compounds for their ability to antagonise the effects of 5-

hydroxytryptamine on the rat uterus. Methyl indoles, methyl-3-indolylacetonitrites and carbazole derivatives, were feeble antagonists of 5-hydroxytryptamine. The most active of the compounds were 5- and 6- benzyloxygramine, the next active compound was N-dimethyl tryptamine (3). The effects of benzyloxygramine compounds were like those of dibenamine and lysergic acid diethylamide, which developed slowly and became irreversible. Eventually, the blockade could not be overcome even by enormous doses of 5-hydroxytryptamine. They called this type of block — "unsurmountable". They concluded from the study of compounds related to tryptamine that antagonistic activity was increased by: i) introduction of two methyl groups on the amino group in the side chain;

ii) introduction of a methyl group in the 2-position of indole nucleus (this effect was also noted by Woolley and Shaw (1953) in another series of substituted indoles);

iii) introduction of a benzyloxy group in the 5-position of indole nucleus of gramine.

(They did not study 5-benzyloxy-N-dimethyl tryptamine (32).

Woolley and Shaw (1957b) have prepared and tested a number of 1,2,3,4-tetrahydrocarbazoles with CH_2-NR or -C(=NR)-NH_2 in the 6-position. Most of

these analogues antagonised the effects of 5-hydroxytryptamine on the rat uterus and carotid artery rings. Two of the compounds (the dimethyl amino methyl tetrahydrocarbazole and tetrahydrocarbazole N-phenyl carboxamidine) proved quite effective in preventing the pressor responses to 5-hydroxytryptamine, when given to dogs intravenously, but were of no such effect when fed by mouth. Dogs and mice receiving a daily dose of 25 mg./kg. of 9-benzyl-6-dimethylaminomethyl-1,2,3,4-tetrahydrocarbazole exhibited excitement within a few days.

Gyermek (1955) and Gyermek, Lazar and Csak (1956) investigated the antagonism of effects of 5-hydroxytryptamine by chloropromazine, phenergan and diparcol on the rat uterus and arterial pressure of decapitated cats. They found that their antagonistic potency ran parallel with their sedative effect. Chloropromazine was the most active antagonist and diparcol the least active.

Berger, Campbell, Handley, Ludwig and Lynes (1957) noted that chloropromazine, reserpine and benactyzine antagonised the effects of 5-hydroxytryptamine on the rat's colon but they also antagonised the effects of acetylcholine as well.

Philippot and Dallemagne (1956) have shown that 5-hydroxytryptamine re-establishes neuro-

muscular transmission in cats blocked by tubocurarine and suggested that tubocurarine might be an antagonist of 5-hydroxytryptamine.

Stimulant analogues of 5-hydroxytryptamine

(i) Tryptamine. This substance was obtained by the action of putrifying bacteria on tryptophan, (Ewins and Laidlaw, 1910), and was studied by Laidlaw (1912). He suggested that it acted on some peripheral nervous structure probably a peripheral neurone and observed the similarity between the action of tryptamine and nicotine on cats' uterus. Tryptamine was used as an ecbolic (Akimoto, 1937) under the name of rutamine. These results have been confirmed by Reid (1951). Raymond Hamet (1941a) observed that the pressor response of tryptamine was reduced by yohimbine. Tryptamine and 5-hydroxytryptamine have been shown to have similar stimulant actions except that tryptamine failed to release adrenaline from the adrenal medulla when injected into the stump of the superior mesenteric artery (Reid, 1952).

Woolley and Shaw (1952) suggested that in the sheep's carotid artery rings 5-hydroxytryptamine acted on receptors which were more "stable" than those acted on by tryptamine. In experiments on the blood pressure of anaesthetised dogs they

obtained further evidence (Woolley and Shaw (1957c) that 5-hydroxytryptamine and tryptamine act on separate receptors. Two substances, "BAS" and "BAS Phenol" blocked the pressor effect of 5-hydroxytryptamine without affecting the responses to tryptamine.

Gaddum (1953a) concluded that on the guinea pig ileum tryptamine and 5-hydroxytryptamine acted on the same receptors. The "M" and "D" receptors of the guinea-pig ileum described by Gaddum and Picarelli (1957, see also page 21) are both regarded as tryptamine receptors.

Vane (1959) showed that on the rat fundus strip, amine oxidase inhibitors (such as Marsilid) potentiated the effects of tryptamine but not those of 5-hydroxytryptamine or of other 5-hydroxy analogues of tryptamine. He showed that suspensions of finely ground rat fundus oxidised tryptamine and 5-hydroxytryptamine. He suggested that, in the isolated preparation, the amine oxidase was unable to inactivate 5-hydroxytryptamine but could inactivate tryptamine, 5-methoxytryptamine and other analogues lacking the 5-hydroxy group. He supposed that tryptamine entered the cell (inside which the amine oxidase was located) whereas 5-hydroxytryptamine and other 5-hydroxy compounds could not do so because of their polar hydroxy group.

(ii) Tryptamine derivatives. N-Methyl tryptamine (13) was found by Chen and Chen (1933) to be less active than tryptamine in raising the blood pressure of a decerebrate cat but more active than N-dimethyl tryptamine (3). N-trimethyl tryptamine (the quaternary salt) was the most active, having 1/20 potency of adrenaline.

Seki (1929) synthesised α-methyl-tryptamine (11) and found that it was as powerful a uterine stimulant as tryptamine but weaker in its action on the heart and intestine.

Bufotenine (21), a substance found in the skin glands of the toad bufo vulgaris (Phisalix and Bertrand, 1893) was shown by Wieland, Konz and Mittasch (1934) to be N-dimethyl-5-hydroxytryptamine. It was synthesised by Hoshino and Shimodaira (1935) and studied by Raymond-Hamet (1941b, 1942a, b and c) and Erspamer (1946). The corresponding quaternary hydroxide, cinobufotenine, was isolated from the Chinese drug "Chan Su" and from secretion from toad's skin (Chen, Jensen and Chen, 1931).

According to Erspamer (1954), if the activity of 5-hydroxytryptamine on the rat uterus is taken as one, then:- bufotenine (21) is 1/10th;
N-methyl 5-hydroxytryptamine 1/3;
5-hydroxy 3-dibutylamino ethyl-indole (25) as 1/1000;
5-methoxytryptamine (28) 1/4;
tryptamine 1/100;
N-methyltryptamine (13) 1/200 and
N-dimethyltryptamine (3) 1/200.

Gaddum et al (1955) found that bufotenine (21) had 1/15th to 1/40th the activity of 5-hydroxytryptamine on the rat uterus. Cinobufotenine (26) was 1/10th as active as 5-hydroxytryptamine on both the rat uterus and guinea pig ileum and they suggested that it acted on both 5-hydroxytryptamine and nicotine receptors on the latter preparation. 5-Methyltryptamine (30), α-methyltryptamine (11) and N-isopropyltryptamine were only 1/500 - 1/1,500 times as active on 5-hydroxytryptamine on the rat uterus.

Cinobufotenine (26) was approximately one half as active as 5-hydroxytryptamine and qualitatively gave similar responses except in cats, where it was usually pressor, whereas 5-hydroxytryptamine was depressor (Page and McCubbin, 1953a). The pressor effect of cinobufotenine (26) in dogs was blocked by yohimbine (Raymond-Hamet, 1943). It was 1/20th to 1/100th times as active as 5-hydroxytryptamine in its ability to stimulate the isolated heart of a mollusc, venus mercenaria (Twarog and Page, 1953), and is reported to be an inhibitor of cholinesterase (Sobotka and Antopol, 1937).

Fabring and Hawkins (1956) reported that intravenous injection in man of bufotenine (26) caused hallucinations and that in monkeys it and lysergic acid diethylamide, produced a syndrome characterised by gross sensory disorders without any marked defect

in the muscular power (Ewart et al. 1955).

Szara (1956, 1957) has shown that N-dimethyltryptamine (3) and N-diethyltryptamine (4) produce psychic effects in man somewhat similar to that of mescaline and lysergic acid diethylamide. A striking difference between the effects of these drugs and those of lysergic acid diethylamide is that with N-dimethyl- and N-diethyltryptamine (3,4) the symptoms appear within 3-4 mins. of the injection of the drug and pass away within 1 hr. whereas the effects of lysergic acid diethylamide take 1-2 hrs. to develop. This may indicate a different mechanism of action. Unlike lysergic acid diethylamide and mescaline, both the derivatives of tryptamine caused choreiform athetoid movements. This phenomenon could be a new tool for investigating experimentally the mechanism of extra-pyramidal compulsive movements. The psychic effects of tryptamine derivatives supports the idea that Schizophrenia may be the result of an abnormal indole metabolism of the body.

According to Erspamer (1954) tryptamine is converted in the body into indole acetic acid (in moderate amounts) and indole aceturic acid (in large amounts). 5-Methoxytryptamine (28) yields 5-methoxyindole acetic acid and N-dimethyltryptamine (3)

yields small amounts of indole acetic acid and moderate amounts of indole aceturic acid. Szara (1956) found that the main metabolite of N-dimethyltryptamine (3) is 3-indole acetic acid.

Conclusion

From what has been described it will be seen that the mode of action of 5-hydroxytryptamine and of its antagonists in the body, is complicated and not yet fully understood. It is, at any rate, certain that 5-hydroxytryptamine has some important function in the body. The properties of analogues of 5-hydroxytryptamine will depend on whether these drugs act like 5-hydroxytryptamine or antagonise it, and also on their ability to cross the blood brain barrier and their susceptibility to attack by such enzymes as amine oxidase. Although it is not possible to predict what substances might have particular effects, there is, nevertheless, a reasonable expectation of finding interesting compounds amongst analogues of 5-hydroxytryptamine.

This thesis describes the actions of substances (many of these new) which modify the actions of 5-hydroxytryptamine. The chief object of this work was to find a potent antagonist of 5-hydroxytrypta-

mine but tests were not restricted simply to antagonistic activity and an attempt has been made to assess their other properties, particularly stimulant activity.

SECTION II

EXPERIMENTS.

EXPERIMENTS

Introduction

It was originally hoped that the compounds would be antagonists of 5-hydroxytryptamine and it was, therefore, expected that tests of antagonistic activity would be done on the rat uterus in the manner described by Gaddum, Hameed, Hathway and Stephens (1955). The first experiments, however, showed that most of the compounds, even those which antagonised 5-hydroxytryptamine, would themselves cause contractions. It was therefore necessary to evolve a quantitative method for determining the stimulant activity. It was planned to use the guinea pig ileum in addition to the rat uterus for determining antagonistic or stimulant properties, but it was first necessary to show that Gaddum and Picarelli's (1957) classification of the tryptamine receptors in the guinea pig ileum into "M" and "D" really applied to tryptamine as well as to 5-hydroxy-tryptamine (see p. 42). This led to a study of the effects of drugs on the responses of the guinea pig ileum to tryptamine and 5-hydroxytryptamine. Some quantitative work was done on the activity of the compounds on the guinea pig ileum, both as antagonists and as stimulants, but the rat fundus strip, which had just been introduced by Vane (1957),

appeared to be more likely to give reliable information, so systematic testing was done on this preparation. The quantitative methods on all these tissues were essentially the same. Some qualitative work was also done on the perfused rabbit's ear and even here the procedure was similar in principle to the qualitative experiments done on the rat uterus, guinea pig ileum and rat fundus strip.

The experimental section is, therefore, divided into: 1) Description of the preparations.

2) Methods: a) General qualitative procedures.

b) Quantitative determination of antagonistic effects.

c) Quantitative determination of stimulant effects.

d) Special procedures for the guinea-pig ileum.

e) Special procedures for the perfused rabbit ear.

1) Preparations

The isolated rat's uterus in oestrus: This tissue was used by Erspamer (1940) for the assay of enteramine (5-hydroxytryptamine) in tissue extracts. In order to increase the sensitivity, Erspamer (1942, 1952) subsequently used ovariectomised rats brought into oestrus by injection of oestradiol. Amin, Crawford and Gaddum (1954) simplified the technique by using normal virgin rats (160-200g.wt.) in which the uterus had been brought into oestrus

by subcutaneous injection of 10 µg. of stilboestrol in 0.1 ml. of arachis oil per 100 g. body weight, the day before the experiment.

Technique: The procedure followed was that of Amin, Crawford and Gaddum (1954). The animal was killed by a blow on the head and both horns of the uterus were dissected out from the adipose tissue and cut into two (making four pieces in all). One piece was used at a time and the rest could be stored in the refrigerator at 4°C for up to 2 days without losing any sensitivity for 5-hydroxytryptamine. The tissue was suspended in De Jalon solution[1] in a 2 ml. bath. One end was fixed and the other tied to a frontal writing lever. The bath temperature was kept at 30°C and compressed air was bubbled through the solution in the bath. The contractions were recorded on a smoked drum with the lever having 1:8 magnification, and the tension on the preparation was 500-750 mg. The tissue was allowed to relax for about 30 min. before any drugs were added. There was a tendency for the responses to be almost all or none with 5-hydroxytryptamine,

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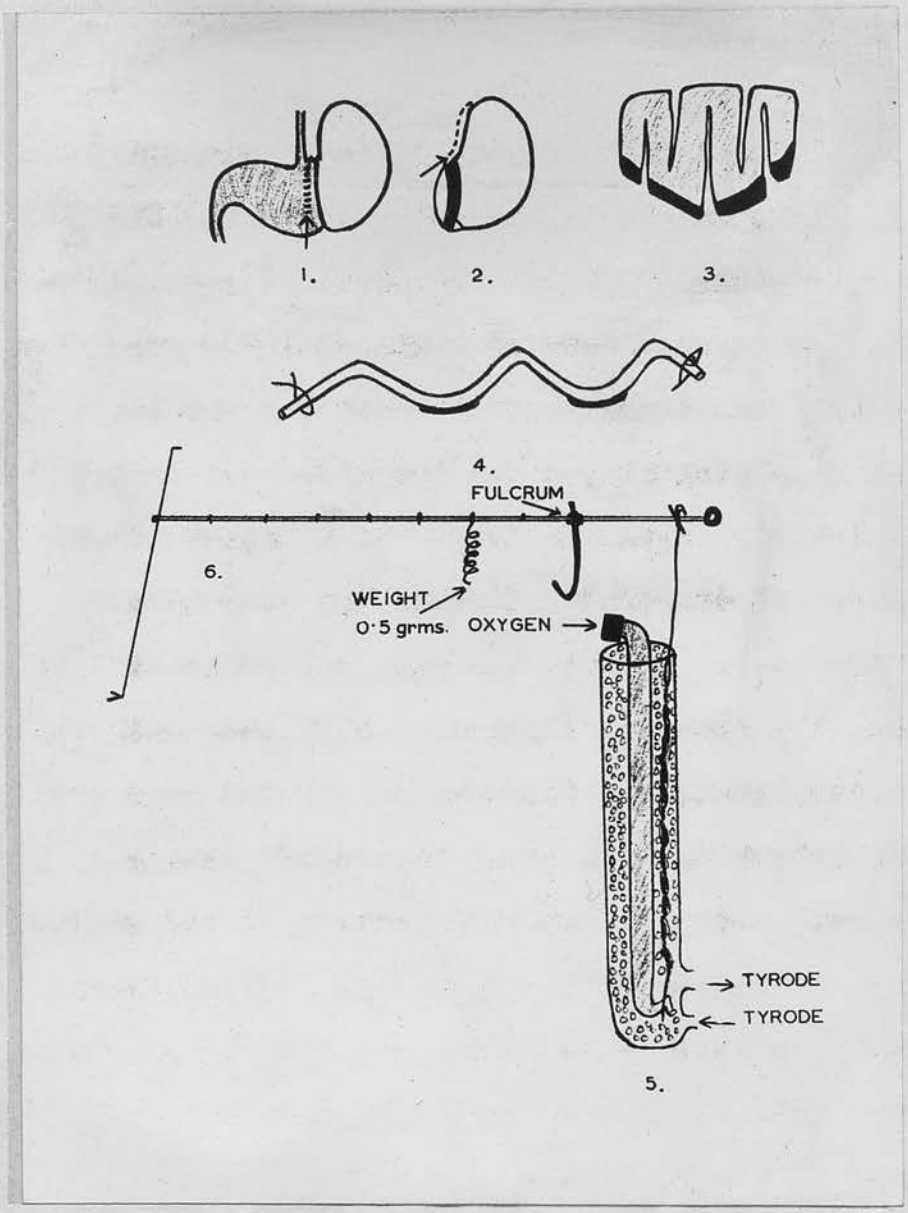
[1]Composition of De Jalon Solution recommended by Gaddum, Peart and Vogt (1949) in gram/litre: NaCl,9; KCl,0.42; $CaCl_2$,0.06; $NaHCO_3$,0.5; Glucose, 0.5.

i.e. the dose response curve was very steep. Increasing the tension on the tissue and/or increasing the magnification of the lever seemed to decrease the slope of the dose response curve. Doses of the order of 5-20 ng. 5-hydroxytryptamine and 1-5 µg. tryptamine usually produced good contractions.

With both the drugs there was a latent period of 15-20 seconds before the start of the contraction and another 30 sec. before it was complete. The bath was then emptied and refilled twice and the muscle then rapidly relaxed (unlike the rat fundus (p. 53). The tissue did not usually show tachyphylaxis to 5-hydroxytryptamine and tryptamine, but to exclude this phenomenon a small dose of 5-hydroxytryptamine was applied frequently in between the different doses of the stimulant drugs. If the response to this dose was depressed, it was important to wait till it recovered before the assay was continued.

The isolated rat fundus strip preparation was described by Vane (1957) who used it for the assay of 5-hydroxytryptamine. He considered it robust and showed that the sensitivity to 5-hydroxytryptamine was 10-100 times more than that of the rat uterus in oestrus. Vane (1959) modified his original technique by recording movements of the muscle, not with a spring lever, but with a pendular auxotonic lever (Paton, 1957).

Figure II



Diagrammatic representation of the rat fundus strip preparation. 1. Stomach as a whole. The arrow is pointing at the cut made in the pyloric part. 2. Isolated fundus along with a thin rim of pyloris. Arrow is pointing at the incision to be made along the lesser curvature to cut open the fundus. 3. The fundus cut open by multiple incisions. 4. The fundus strip tied and pulled out. The bits of the pyloric tissue are to be trimmed off. 5. The long bath holding the tissue and the tube supplying oxygen. 6. Auxotonic pendular lever (Paton, 1957

Technique: A rat of 250-400 g. was killed by a blow on the head and the fundus of the stomach removed. (There is a clear dividing line between the white fundus part and the pink pyloric part). It was washed with tyrode[1] solution and cut into a long strip exactly as described by Vane (Fig. II). The strip was suspended in tyrode solution at 37°C in a long narrow bath of 8 mm. diameter and 15 cm. length; the tissue was fixed at one end to the tube supplying oxygen and the other end attached to the lever. Normally there was a steady flow of tyrode 60 drops/min. through the bath (overflowing at the top). Before the drug was added to the bath, the flow was stopped. When the contraction was completed, the flow was re-started at a rapid rate (about 2.5 ml./sec.) for 10-15 seconds to wash the drug out; it was then reduced to the original rate. The movements of the muscle were recorded with a calibrated pendular auxotonic lever (Paton, 1957) made of balsa wood with a frontal writing point on a smoked drum.

In some preliminary experiments the lever, like that used by Vane, had a magnification of 1:16 and the load on the muscle was 1 gram. In order

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[1]Composition of tyrode solution in gram/litre: $NaCl$, 0.8; $CaCl_2$, 0.02; KCl, 0.02; $MgCl_2$, 0.01; NaH_2PO_4, 0.005; $NaHCO_3$, 0.1; Glucose, 0.1.

to plot the entire dose/response curve with the stimulant compounds, only three-quarters of the length of the dissected tissue was used; the magnification of the lever was reduced to 1:4 and the load on the muscle was 0.5 gram. The tissue was allowed to relax for about 45 min. before the start of the experiment. The drugs were added to the bath with a syringe in a volume not exceeding 0.2 ml. The tissue was stretched after each contraction with 5-hydroxytryptamine and tryptamine for a period of 60 sec. by bringing down the lever to a fixed level just below the normal base line. Such treatment did not disturb the regularity of the base line. Air was bubbled through a test tube holding water which was attached to the bar supporting the lever. It caused sufficient vibration to prevent the writing point sticking. Hyoscine hydrobromide was added to the tyrode solution in a concentration of 100 ng./ml. as a routine, except when atropine-like drugs were investigated (p.120). This drug reduces any acetylcholine-like effects of the compounds, and also the irregularity of the base line. The contractions caused by 5-hydroxytryptamine were complete in about 90 sec. and the preparation was then washed for 10-15 sec. and, starting at the same time, stretched for about 60 sec. A further 2½ min. were allowed for the recovery with a complete cycle of 5 minutes.

The isolated guinea pig ileum: The action of 5-hydroxytryptamine on this tissue has been studied by Robertson (1953), Rocha e Silva, Valle and Picarelli (1953), Gaddum (1953a) and Gaddum, Hameed and Hathway and Stephens (1955). The preparation has been used for the assay of 5-hydroxytryptamine and for the study of drugs which modify the action of 5-hydroxytryptamine. Gaddum and Picarelli (1957) devised a technique for investigating the effects of different drugs on the "D" and "M" type of 5-hydroxytryptamine receptors (p.21). When a concentration of 1 µg./ml. morphine is maintained in the bath, the "M" receptors appear to be more or less completely blocked, and any additional antagonistic effect of a drug is then thought to be due to an action on the "D" receptors. When the intestine is exposed to a concentration of 0.1 µg./ml. dibenzyline for 30 min. the "D" receptors appear to be more or less completely and permanently blocked and any additional effect is then thought to be on the "M" receptors.

When investigating the effect of drugs on the guinea pig ileum, it is, therefore, usual to use the preparation treated with morphine (for the action on "D" receptors) or the one treated with dibenzyline (for the action on "M" receptors).

Technique: Guinea pigs weighing 150-260 g. which had been deprived of food over-night, were killed by a blow on the neck and by cutting the throat. The portion of ileum nearest to the ileo-caecal junction was removed and cleaned with tyrode solution, introduced into the lumen by a pipette if necessary. A 2 cm. piece of gut was tied at both ends with cotton and suspended in a 2 ml. bath in tyrode solution at 37°C. Compressed air was bubbled through this bath at a moderate speed. The movements of the gut were recorded with a light frontal writing lever with 1:5 magnification on a smoked drum.

The dose of 5-hydroxytryptamine used was in the range of 10-100 ng. and of tryptamine in the range of 1-10 µg. In order to obtain a quiet and sensitive preparation, it is important to avoid un-necessary trauma to the tissue. If the tissue were left in tyrode solution at room temperature for more than 1 hour, its sensitivity decreased. 5-hydroxytryptamine and tryptamine contractions were completed within 30-45 sec. and after being washed twice the preparation relaxed rapidly. In order to avoid interference from tachyphylaxis, a small dose of 5-hydroxytryptamine was applied in between the different stimulant doses and the experiment was continued only when these responses were normal. The drugs were added in a volume of up to 0.2 ml.

to the bath, and the cycle of events took 4 minutes.

In the study of drugs on the "D" and "M" receptors, the technique used was that of Gaddum and Picarelli (1957). Morphine in a concentration of 3.5×10^{-6} M was added to the tyrode solution, so that the tissue was constantly exposed to the action of morphine, when drugs were to be tested on the "D" receptors. Similarly, when used for investigating the effect of drugs on the "M" receptors, the tissue was exposed to 3.4×10^{-7} M dibenzyline for 30 min. at the beginning of the experiment, alternatively lysergic acid diethylamide was added to the tyrode solution in a concentration of 3×10^{-8} M.

In the presence of morphine, small responses were produced by about 10 ng. 5-hydroxytryptamine or 1 µg. tryptamine; maximum responses were produced by 100 ng. 5-hydroxytryptamine or 10 µg. tryptamine. After the ileum had been treated with dibenzyline small responses were produced by about 500 ng. 5-hydroxytryptamine and maximal responses by 4 µg. 5-hydroxytryptamine. A 200 µg. dose of tryptamine only produced a threshold response. When lysergic acid diethylamide was maintained in the tyrode solution, contractions were caused by doses of between 200 ng. and 2 µg. 5-hydroxytryptamine and by between 10 µg. and 200 µg. tryptamine.

Responses to 5-hydroxytryptamine and tryptamine on the guinea pig ileum in the presence of morphine

and lysergic acid diethylamide, and after treatment with dibenzyline, appeared to be the same as in the normal guinea pig ileum, in that the contractions were completed within 30-45 sec. of the application of the drug, and there was a rapid relaxation after washing out the 5-hydroxytryptamine and tryptamine. The time interval between the application of the different stimulant drugs was still maintained at 4 minutes. A dose of 5-hydroxytryptamine causing a small contraction was added at intervals to detect tachyphylaxis.

The perfused rabbit's ear: This tissue was used by Rapport, Green and Page (1948) for the study of serotonin, and also studied by Fingl and Gaddum (1953), Gaddum and Hameed (1954), Garven (1955) and Savini (1956).

Technique: A rabbit, preferably with large veins in the ears, was killed by a blow on the head, care being taken to avoid injury to the ears. Both ears were cut off as close to the skull as possible. The central artery, vein and nerve lying on the posterior aspect of the pinna near its thinner margin were dissected out. The skin overlying these vessels was slit longitudinally to get a good view. A thread was tied round the tip of the artery, which looks whiter than the vein. A small polythene cannula was inserted into the artery. Some Green

and Page solution[1] was cautiously injected through the cannula with a syringe. If the procedure so far were correct, blood-coloured fluid should come out of the main vein lying near the cannulated artery. The polythene cannula was fitted to a Gaddum and Kwiatkowski's (1938) rubber-capped injection cannula and was connected to the perfusion reservoir containing Green and Page solution. The ear was placed on a draining plate and fixed with a pin. The perfusate coming out of the cut veins was collected in a funnel which ended in a horizontal capillary tube. The capillary end of the collecting funnel was then attached to a drop timer (Gaddum and Kwiatkowski, 1938). The reservoir containing Green and Page solution had a capacity of 2 l. and was kept at a distance of about 12 in. from the level of the pinna, in these circumstances the flow through the ear should be 60-70 drops/min. The tube connecting the reservoir to the injection cannula must be left without any obstruction, to avoid any intervention by a second constriction in the passage of the solution. The reservoir should not be raised (to increase the flow) more than a few inches at a time or the tissue may become permanently damaged. The fluid was allowed to flow through the ear for a few minutes till the perfusate was clear. The ear was then removed (together

with the cannula attached to its artery) and kept in normal saline at 4°C over-night. The tissue was very sensitive the next day and could be used continuously until the third day. When the switch of the drop recorder was at "make", the time the drop took to stay in between the two electrodes corresponded with the vertical height of the record of the drum.

The drugs were injected through the injection cap into the air space very slowly, to avoid altering the pressure of the perfusion fluid. The interval between the application of different stimulant drugs depended on the degree of vasoconstriction produced. An interval of 3-10 min. was sufficient between the different doses which produced moderate responses. The drugs were injected up to a final volume of 0.1 ml. When a large dose of the stimulant drug was given and the resultant vasoconstriction was severe, the recovery could be hastened by raising the reservoir by a few inches for a short time. Convenient doses (which produced moderate effects) were 0.5-1 ng. 5-hydroxytryptamine, 5-10 ng. tryptamine and 2.5-5 ng. adrenaline.

2. Methods

a) General qualitative procedures: The purpose of this type of experiment was to find out in a qualitative manner what the drugs did. A few

responses to 5-hydroxytryptamine were obtained and a suitable dose giving a small response was selected, about 5 ng. in the rat uterus and rat fundus strip. This was given at regular intervals throughout the experiment. The test drug was applied to the tissue in a low concentration (about 10^{-7} M on the rat uterus and fundus) and washed twice (after 45 sec. on the rat uterus and after 90 sec. on the rat fundus). The selected dose of 5-hydroxytryptamine was then given to see if the test drug had any effect, such as antagonism or synergism. If the response to the selected dose of 5-hydroxytryptamine were altered, the dose was repeated at the usual interval (with washing) until the response returned to normal. The test drug was then again applied, but in 10 times the previous concentration, and the experiment continued. The highest concentrations tested were 10^{-5} to 10^{-4} M. In some experiments with very high concentrations, the drugs were left to act for 10-15 minutes.

b) Quantitative determination of antagonistic activity:

i. Determination of the dose ratio: The dose of 5-hydroxytryptamine (or tryptamine or acetylcholine) which caused about 50% of the maximum response was found; to do this it was usually necessary to apply two or three different doses of 5-hydroxytryp-

tamine in order to obtain some idea of the dose response curve. After this, the antagonist was maintained in the bath for a particular period (usually 1 hr.) and the dose of 5-hydroxytryptamine (or tryptamine or acetylcholine) which caused 50% of the maximum response was again determined. The ratio of the two concentrations, after and before the action of the antagonist, is the dose ratio for that particular concentration of antagonist applied for that particular time. In some cases the experiment was continued, and the recovery of the tissue observed after the antagonist was removed. (On the rat uterus, it was possible to add the antagonist directly to the bath, but on the rat fundus strip the antagonist had to be added to the reservoir containing tyrode solution, which flowed over the preparation). As the dose ratio varies with the concentration, it can only be used to compare the antagonistic activity of substances which are active at similar concentrations. In order to assess with any accuracy the relative potency of substances which differ in activity to any extent, it is necessary to use the drug ratio described below.

ii. Determination of the drug ratio: A few doses of the stimulant drug (always 5-hydroxytryptamine) were given, in order to find the doses which cause a maximum response and 50% of the maximum

response.

The antagonist was maintained in the bath in a suitable concentration, previously determined by the dose ratio experiments. When the antagonist was present, more agonist was required in order to produce a response 50% of the maximum, and at the end of 1 hr. the concentration of stimulant which produced 50% of the maximum response of the tissue was determined. The drug ratio was obtained by dividing this concentration by the concentration of the antagonist. It should be independent of the concentration of the antagonist, because if there is more antagonist present, more stimulant drug will be required. The drug ratio is, therefore, a convenient measure of antagonistic activity. In point of fact, with many drugs the block produced by higher concentrations could not be overcome by increasing the concentration of the stimulant drug. This form of irreversible block has been referred to as "unsurmountable" (Gaddum, Hameed, Hathway and Stephens, 1955).

To check that the antagonism is specifically against 5-hydroxytryptamine, control contractions, usually with acetylcholine, were obtained before and after the antagonist was given. Gaddum, Hameed, Hathway and Stephens (1955) have stated that this method is unlikely to give reliable

results when the dose ratio is small, and the concentration of the antagonist should therefore be always high enough to produce a dose ratio of at least 5 and generally much more. On the rat uterus antagonist activity was measured by means of the drug ratio whenever possible but on the rat fundus strip this was not done even when the dose ratios were more than 5. The production of a maximal response on the rat fundus, which is essential for determining the drug ratio, would render the tissue insensitive for a considerable time afterwards.

PA_2 values (Gaddum, Hameed, Hathway and Stephens, 1955) of the antagonists were not determined because the activity of the compounds was not very high.

c) Quantitative determination of stimulant activity: The log. dose/response curves of 5-hydroxytryptamine and one of the stimulant compounds were compared on one tissue. These curves (Fig. were plotted and examined with the naked eye to see if they were parallel. If they were, it was possible by measuring the distance between the two lines, to determine the equipotent molar ratio of the test compound, that is, the number of molecules of the drug required to produce the same effect as that of one molecule of 5-hydroxytryptamine (Table I). A high figure indicates a low potency.

In these experiments, it was necessary with some of the compounds to modify the cycle of events. On the rat uterus it was a long time before the effects of certain compounds wore off, and the control response with the small test dose of 5-hydroxytryptamine returned to normal. On the rat fundus, it was sometimes necessary to allow up to 3 min. for the contraction to reach its maximum, and also to allow even up to 45 min. stretching to assist recovery. If insufficient time was allowed for recovery, i.e. for the base line to return to normal and the control response to 5-hydroxytryptamine to recover, the responses to the test drug were reduced and the dose response curves consequently appeared to flatten out. This gave a misleading impression in the earlier part of the work, that these compounds were partial agonists (Stephenson, 1956). Later on, substances were found which genuinely showed characteristic flattening out of their dose response curves below the top. This occurred even though the control responses to 5-hydroxytryptamine indicated that the preparation had been allowed sufficient time for recovery.

In stimulant experiments on the rat fundus it was necessary to reduce the magnification of the lever to 1-4 (see p.53), in order to record the

maximum responses. Because of the persistence of the effects of high doses, the doses were given in increasing order of magnitude, rather than in any random order. After many of the compounds, the rat uterus only returned to normal very slowly, and the determination of their dose/response curves became very tedious, particularly with high doses. With such compounds, simple matching experiments were performed and the equipotent molar ratios calculated by comparing the doses of 5-hydroxytryptamine and the test drug which produced roughly 50% of the maximum contraction (these are indicated by "M" in Table I).

d) Special procedures for the guinea pig ileum.

It was first necessary to compare the dose ratios of a number of standard antagonists against 5-hydroxytryptamine with the dose ratios against tryptamine. The antagonists used were lysergic acid diethylamide and morphine, in concentrations identical with those used by Gaddum and Picarelli (1957). Acetylcholine and nicotine were used as control drugs. Although it was not necessary in determining the dose ratio to plot the complete dose response curve for 5-hydroxytryptamine and tryptamine, this was, nevertheless, done both before and after the action of lysergic acid diethylamide

The results (which are discussed on p. 95 indicated that Gaddum and Picarelli's (1957) assumptions were justified, and this preparation was therefore used for testing both antagonistic and stimulant activity of a number of the compounds. Exactly the same procedure was used as in the experiments on the rat uterus and the rat fundus strip, except that the tyrode solution contained a concentration of either morphine (3.5×10^{-6} M) or lysergic acid diethylamide (3×10^{-8} M). Experiments were also done on the preparation treated with dibenzyline. In these, the tissue was exposed to a concentration of dibenzyline (3.4×10^{-7} M) for half-an-hour. The preparation was then washed and used with normal tyrode. After this treatment with dibenzyline, regular responses could only be obtained with 5-hydroxytryptamine for about 2 hr. and in any experiment it was only possible to compare the stimulant properties of one compound with those of 5-hydroxytryptamine. The other two preparations (in the presence of lysergic acid diethylamide and morphine) were, however, strong enough to allow more than one such comparison between 5-hydroxytryptamine and an analogue.

e) <u>Special procedures for the perfused rabbit ear</u>: If the general qualitative procedure showed that a compound antagonised the effects of 5-hydroxytryptamine on this preparation, the following pro-

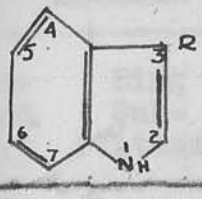
cedure was adopted. Equivalent responses were obtained with 5-hydroxytryptamine and adrenaline. A dose of the compound which was expected to antagonise 5-hydroxytryptamine (from the qualitative experiment), was given and followed by the original doses of 5-hydroxytryptamine, tryptamine and adrenaline. If there was antagonism, the doses of the stimulant drugs were increased in order to obtain the dose ratio, but the dose of the antagonist was not repeated and thus the antagonism gradually wore off.

The stimulant activity on this preparation was determined in the usual way, by plotting the dose response curves from the compounds, 5-hydroxytryptamine and tryptamine. This tissue could be used for many comparisons during the day, but the range of concentrations is very limited, because it is very easy to produce the maximum vasoconstriction by merely doubling the dose. Drugs which had no effect in doses of about 1 μ. mol. were regarded as inactive.

SECTION III

RESULTS

TABLE 1



Compounds and their stimulant activity on the rat uterus and rat fundus strip.

Compound No.	Ring Substituent	Side-chain (R) (Trivial names)	Molecular weight	STIMULANT ACTIVITY Equipotent Molar ratios: Mean ± S.E. Rat uterus 30°C	No. of Expt	Rat fundus strip 37°C	No. of Expt
1	5-OH	$-CH_2-CH_2-NH_2$ (5-hydroxytryptamine)	Creatinine sulphate 405	1		1	
2	-	$-CH_2-CH_2-NH_2$ (tryptamine)	HCl 196.5	210 ± 30	4	933 ± 33	3
3	-	$-CH_2-CH_2-N(CH_3)_2$ (N-Dimethyltryptamine)	Fumarate 304	† 1000(M)	2	350 ± 100	4
4	-	$-CH_2-CH_2-N(C_2H_5)_2$ (N-Diethyltryptamine)	HCl 252.5	500(M)	2	112 ± 14	3
5	-	$-CH_2-CH_2-N(C_3H_7)_2$ (N-Dipropyltryptamine)	HCl 280.5	200 ± 23	3	40 ± 9	3
6	-	$-CH_2-CH_2-N(iso\ C_3H_7)_2$ (N-diisopropyltryptamine)	HCl 280.5	520 ± 104	3	53 ± 8	4
7	-	$-CH_2-CH_2-N(C_4H_9)_2$ (N-Dibutyltryptamine)	HCl 308.5	500(M)	2	244 ± 65	4
8	-	$-CH_2-CH_2-N$ (N-pyrrolidinotryptamine	HCL 250.5	2000(M)	1	470 ± 115	3
9	-	$-CH_2-CH_2-N$ (N-piperidinotryptamine	HCl 264.5	1600(M)	1	7500 ± 1770	2
10	-	$-CH_2CH_2-N$ (N-morpholinotryptamine	HCl 266.5	>10,000(M)	2	>75,000	2
11 (C)	-	$-CH_2-CH(CH_3)-NH_2$ (α-Methyltryptamine)	HCl 210.5	800	3	50 ± 14	3
12 (C)	-	$-CH_2-CH(C_2H_5)-NH_2$ (α-Ethyltryptamine)	HCl 224.5	>20,000	2	10,000	2
13	-	$-CH_2-CH_2-NH(CH_3)$ (N-Methyltryptamine)	HCl 210.5	2,000(M)	2	2400 ± 98	3

TABLE 1: Contd.

Compound No.	Ring Substituent	Side-chain (R) (Trivial names)	Molecular weight	STIMULANT ACTIVITY Equipotent Molar ratios: Mean±S.E. Rat uterus 30°C	No. of Expt	Rat fundus strip 37°C	No. of Expt
14	-	$-CH_2-CH_2-NH(C_2H_5)$ (N-Ethyltryptamine)	HCl 224.5	2000(M)	2	350 ± 29	3
15	-	$-CH_2-CH_2-NH(C_3H_7)$ (N-Propyltryptamine)	HCl 238.5	2000(M)	2	280 ± 42	4
16	2-CH_3	$-CH_2-CH_2-N(CH_3)_2$ (2-Methyl-N-dimethyltryptamine)	HCl 238.5	>20000(M)	2	6665 ± 1330	3
17	2-CH_3	$-CH_2-CH_2-N(C_2H_5)_2$ (2-Methyl-N-diethyltryptamine)	HCl 266.5	1000(M)	2	280 ± 42	4
18	2-CH_3	$-CH_2-CH_2-N(C_3H_7)_2$ (2-Methyl-N-dipropyltryptamine)	HCl 294.5	630(M)	2	70 ± 10	3
19 (S)	1-CH_3	$-CH_2-CH(CH_3)-NH_2$ (-Methyl-α-methyl tryptamine)	HCl 238.5	1350 ±450 (M)	2	69 ± 14	3
20(C) ((S)	5-OH	$-CH_2CH(CH_3)NH_2$ (5-Hydroxy-α-methyltryptamine)	HCl 226.5 C. sulphate 419	2.0 ± 0	2	1.7 ± 0.2	5
21	5-OH	$-CH_2-CH_2N(CH_3)_2$ (Bufotenine)	Base 204 Fumarate 524	2.7 ± 1.6	3	3.1 ± 1.8	3
22	5-OH	$-CH_2-CH_2N(C_2H_5)_2$ (5-Hydroxy-N-diethyltryptamine)	HCl 268.5	9 ± 2.6	3	18 ± 9.5	3
23	5-OH	$-CH_2-CH_2N(C_3H_7)_2$ (5-Hydroxy-N-dipropyltryptamine)	HCl 296.5	39 ± 1.5	3	16 ± 4.0	6
24	5-OH	$-CH_2-CH_2N$isoPr$_2$ (5-Hydroxy-N-diisopropyltryptamine)	HCl 296.5	16 ± 0	3	4.5 ± 1.5	3
25	5-OH	$-CH_2-CH_2N$n-Bu$_2$ (5-Hydroxy-N-dibutyltryptamine)	HCl 324.8	† 1,111	2	151 ± 40	3

TABLE 1: Contd.

Compound No.	Ring Substituent	Side-chain (R) (Trivial names)	Molecular weight	STIMULANT ACTIVITY Equipotent Molar ratios: Mean ± S.E. Rat uterus 30°C	No. of Expt	Rat fundus strip 37°C	No. of Expt
26	5-OH	$-CH_2-CH_2\overset{+}{N}Me_3\bar{I}$	Iodide 346	>20,000	2	>10,000	2
27	5-OH	$-CH_2-CH_2\overset{+}{N}Me_2Et_1\bar{I}$	Iodide 360	>20,000	2	>10,000	2
28 (Cu)	5-OMe	$-CH_2-CH_2NH_2$ (5-Methoxytryptamine)	HCl 226.5	8 ± 1.7	3	39 ± 9.7	3
29 (V)	5-OMe	$-CH_2-CH(CH_3)NH_2$ (5-Methoxy-α-Methyltryptamine)	HCl 240.5	4.3 ± 1.4	3	1.4 ± 0.3	3
30 (C)	5-Me	$-CH_2-CH_2-NH_2$ (5-Methyltryptamine)	HCl 210.5	173 ± 80	3	620 ± 180	3
31 (C)	5-Me	$-CH_2-CH(CH_3)-NH_2$ (5-Methyl-α-methyltryptamine)	HCl 228.5	1300 ± 17	3	22.7 ± 1.5	4
32	$5-OC_7H_7$	$-CH_2-CH_2-NMe_2$ (5-Benzyloxy-N-dimethyltryptamine)	HCl 330.5	>20,000	2	†625 ± 135	4
33	$5-OC_7H_7$	$-CH_2-CH_2NEt_2$	HCl 358.8	>10,000	1	†470 ± 60	4
34	$5-OC_7H_7$	$-CH_2-CH_2NnPr_2$	HCl 386.5	>20,000	1	†867 ± 183	2
35	$5-OC_7H_7$	$-CH_2-CH_2NisoPr_2$	HCl 386.5	>10,000	1	†614 ± 80	3
36	$5-OC_7H_7$	$-CH_2-CH_2NnBu_2$	HCl 414.8	20,000	1	20,000	1
37	$5-OC_7H_7$	$-CH_2-CH_2N$ 	HCl 372.7	>20,000	1	>5,000	1
38 (S)	$5-OC_7H_7$	$-CH_2-CH(CH_3)NH_2$	HCl 317.5	20,000	1	†63 ± 11	2

TABLE 1: Contd.

Compound No.	Ring Substituent	Side-chain (R) (Trivial names)	Molecular weight	STIMULANT ACTIVITY Equipotent Molar ratios: Mean ± S.E Rat uterus 30°C	No. of Expt	Rat fundus strip 37°C	No. of Expt
39	5-OC_7H_7	$-CH_2-CH_2\overset{+}{N}Me_3\bar{I}$	Iodide 436	> 10,000	2	> 5,000	2
40	5-OC_7H_7	$-CH_2-CH_2\overset{+}{N}Me_2Et\bar{I}$	Iodide 450	> 20,000	2	> 10,000	2
41	5-OC_7H_7	$-CH_2N(CH_3)_2$ (5-Benzyloxy-gramine)	Base 280	NIL (Gaddum et al, 1955)		25,000	1
42 (W)	1-Benzyl-2-methyl-5-methoxytryptamine (Woolley and Shaw's BAS)		HCl 375.5			> 20,000	2

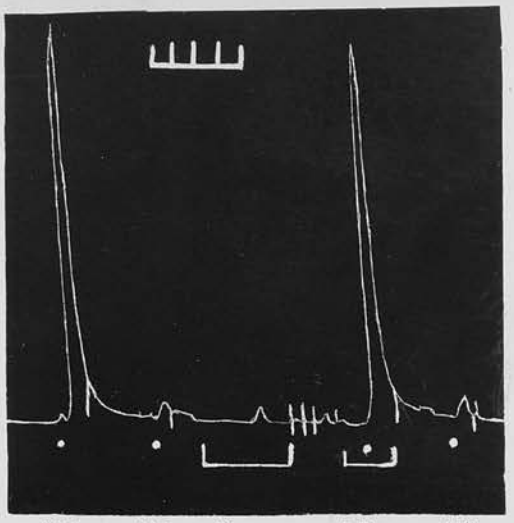
† Indicates the dose/response curve was not parallel to that of 5-hydroxytryptamine, at the top.

(M) indicates that estimates were determined by matching (see text).

The explanation of (S), (C), (Cu), (V) and (W) are given in the acknowledgments.

Figure III (a)

Isolated rat uterus preparation

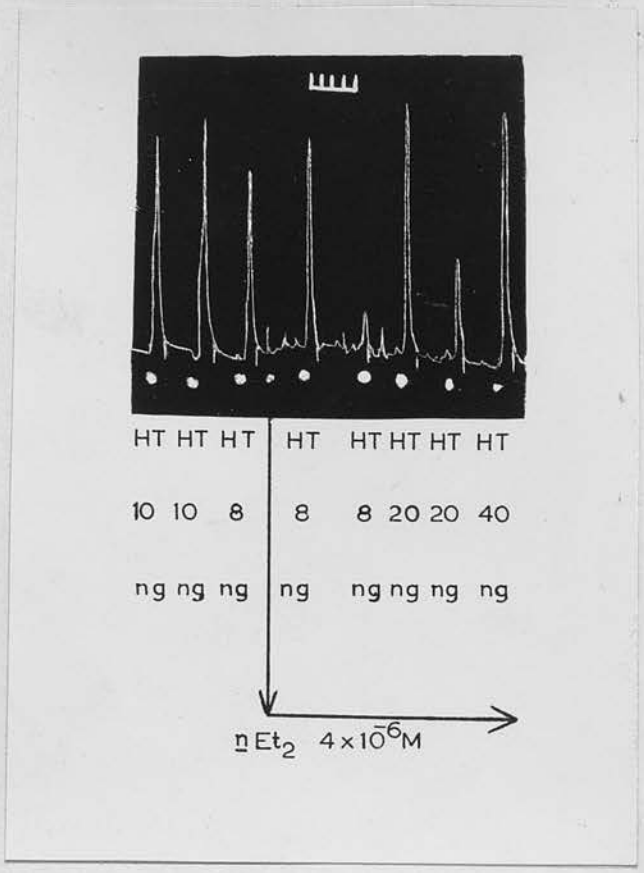


Synergism between 5-hydroxytryptamine (HT) and N-dibutyltryptamine ($\underline{n}Bu_2$). N-dibutyltryptamine (4.8×10^{-6}M) by itself had no effect on the tissue but when added with 2 ng. of HT, the response was about as big as that of 4 ng. of HT, previously.

Time in minutes.

Figure III (b)

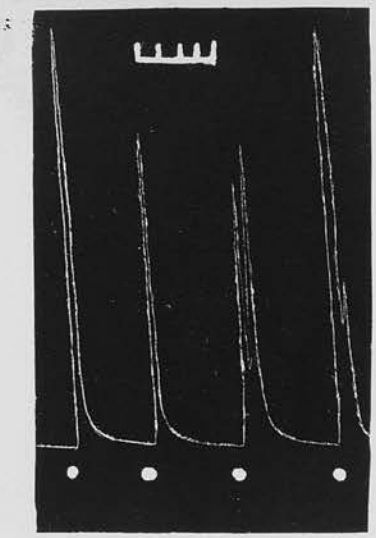
Isolated rat uterus preparation



Antagonism of 5-hydroxytryptamine (HT). Responses are shown by HT (8, 10, 20 and 40 ng.) before and after maintaining 4×10^{-6}M concentration of N-diethyltryptamine ($\underline{n}Et_2$) as indicated. After 18 min. 40 ng. of HT produced a response equivalent to 10 ng. of HT, previously giving a dose ratio of 4.

Figure III (c)

Isolated rat uterus preparation.



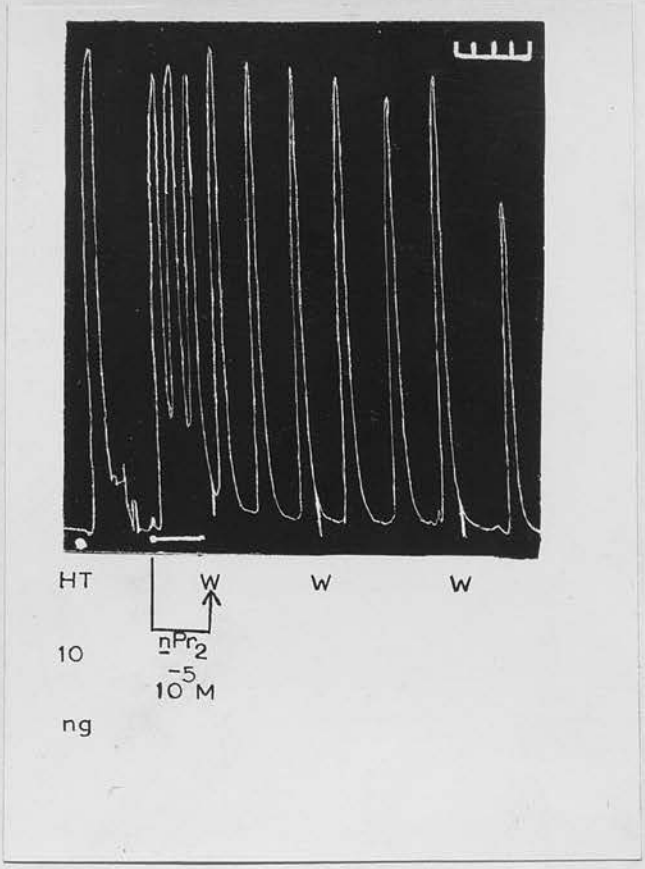
Stimulant action of diisopropyltryptamine ($\underline{i}Pr_2$). 5-Hydroxytryptamine (HT) 5 and 10 ng. gave comparable responses to 3.5 and 7 n. Moles of the analogue.

10 ng. of HT = 5.5 p Moles.

Time in min.

Figure III (d)

Isolated rat uterus preparation



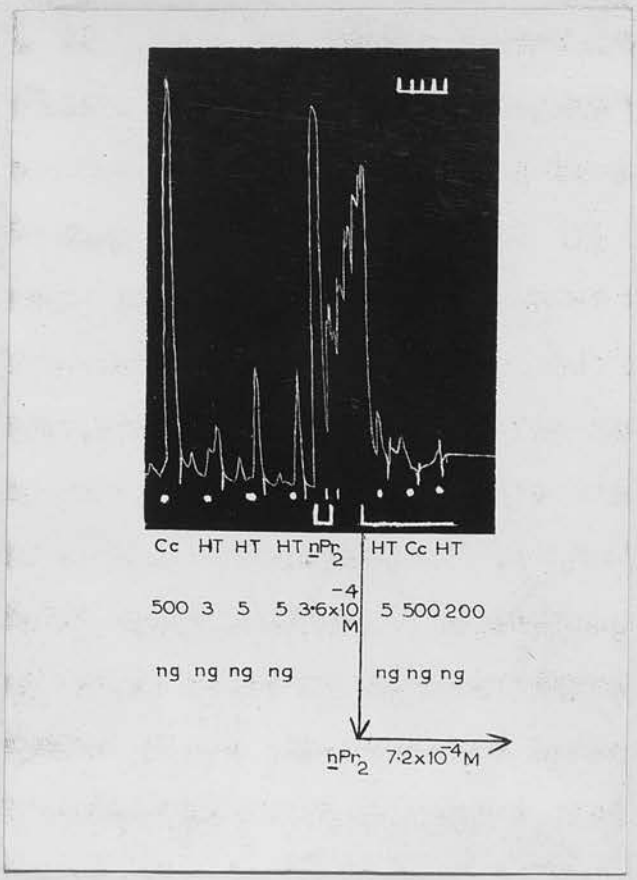
Repeated contractions caused by N-dipropyltryptamine (nPr_2). The analogue, in concentration of 10^{-5}M was maintained in the bath for 4 min. The repeated contractions persisted in spite of repeated washings at W.

HT is 5-hydroxytryptamine.

Time in min.

Figure III (e)

Isolated rat uterus preparation



Profound depression.

Responses to carbachol (Cc 500 ng) and 5-hydroxytryptamine (HT 3, 5 and 200 ng.) are shown before and after treatment of the tissue with dipropyltryptamine (3.6 and 7.2 x 10^{-4} M). After adding the analogue in a concentration of 7.2 x 10^{-4}M, the base line suddenly became steady and both Cc and HT were blocked.

RESULTS

1. EFFECTS ON THE ISOLATED RAT UTERUS

Introduction. The qualitative experiments showed that the effects varied with the concentrations as well as with the nature of the drug. It was found that a few compounds (3, 25, 32-38) would antagonise the effects of 5-hydroxytryptamine, but some of these, (3, 25) when given in higher concentrations, themselves caused contractions of the preparation. Nearly all the remaining compounds caused contractions without antagonising 5-hydroxytryptamine in lower concentrations. Still higher concentrations (10^{-5} M) of any of the compounds which could stimulate the preparation (including those which antagonised 5-hydroxytryptamine in lower concentrations caused repeated contractions, and in even higher concentrations caused profound depression in which the tissue failed to respond even to acetylcholine. Fig.III illustrates all these different types of effect which are divided into:

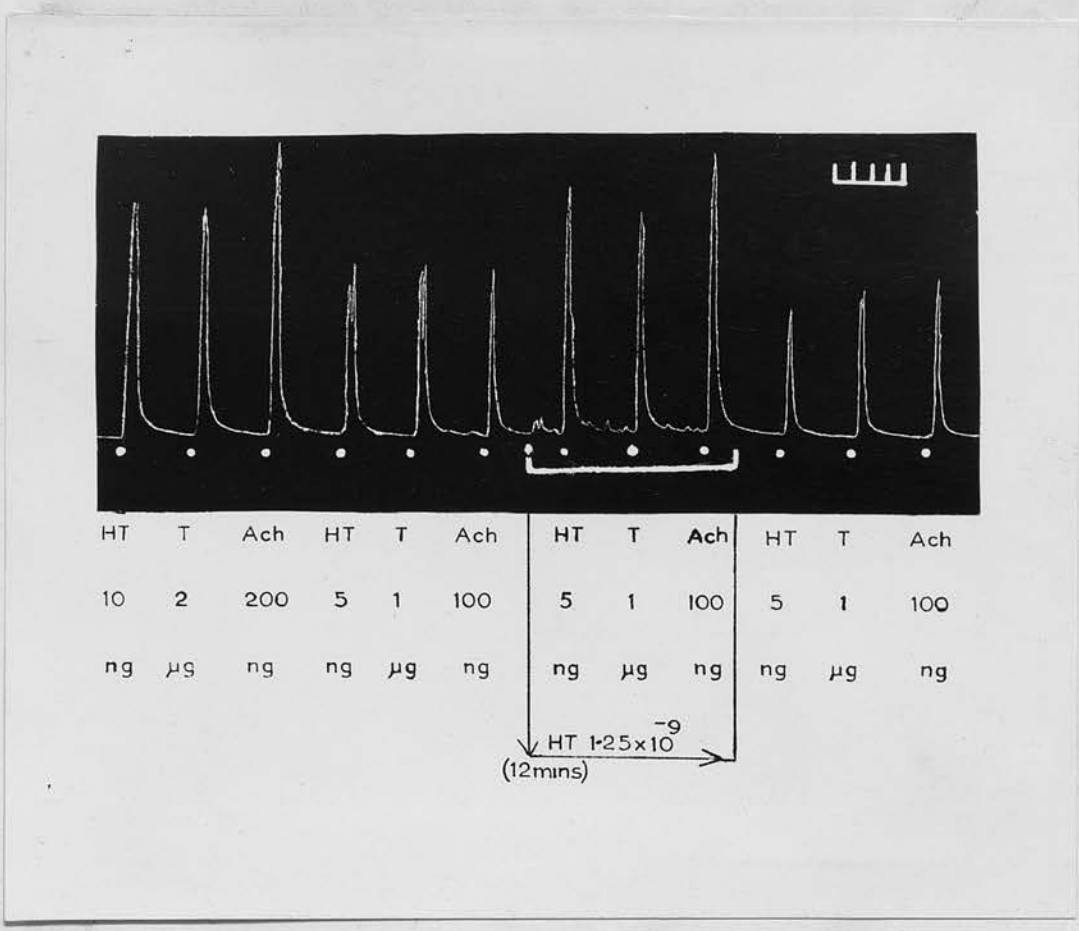
(i) Synergism with 5-hydroxytryptamine, tryptamine and acetylcholine.
(ii) Antagonism to 5-hydroxytryptamine and tryptamine.
(iii) Stimulant action.
(iv) Production of repeated contractions.
(v) Profound depression.

(i) Synergism. It was thought that the compounds might potentiate the actions of 5-hydroxy-

tryptamine, so responses were obtained with these two drugs, and with acetylcholine. The preparation was then treated for up to 30 minutes, with the test drug in a concentration so low that it did not cause contraction. Responses were again obtained with 5-hydroxytryptamine, tryptamine and acetylcholine. All these were often bigger than the original responses, but were never as much as the responses to twice the original dose. This phenomenon did not seem to be potentiation similar to the ten- or more fold increase in acetylcholine responses produced by eserine. It was referred to as 'synergism'. It seemed possible that it might be a form of simple addition, particularly because the dose response curve of 5-hydroxytryptamine in this preparation is very steep. For example, in a particular preparation, although 4 ng. 5-hydroxytryptamine produced a reasonable contraction, 2 ng. 5-hydroxytryptamine had no apparent effect. The addition of 2 ng. 5-hydroxytryptamine to a preparation which had already been given 2 ng. 5-hydroxytryptamine (without apparent effect) might result in a contraction equivalent to that produced by 4 ng. Fig.IIIa shows an experiment in which a low dose (4.8×10^{-6} M) of N-dibutyltryptamine (7) was given; this, by itself, had no effect, but when 2 ng. 5-hydroxytryptamine was

Figure IV

Isolated rat uterus preparation

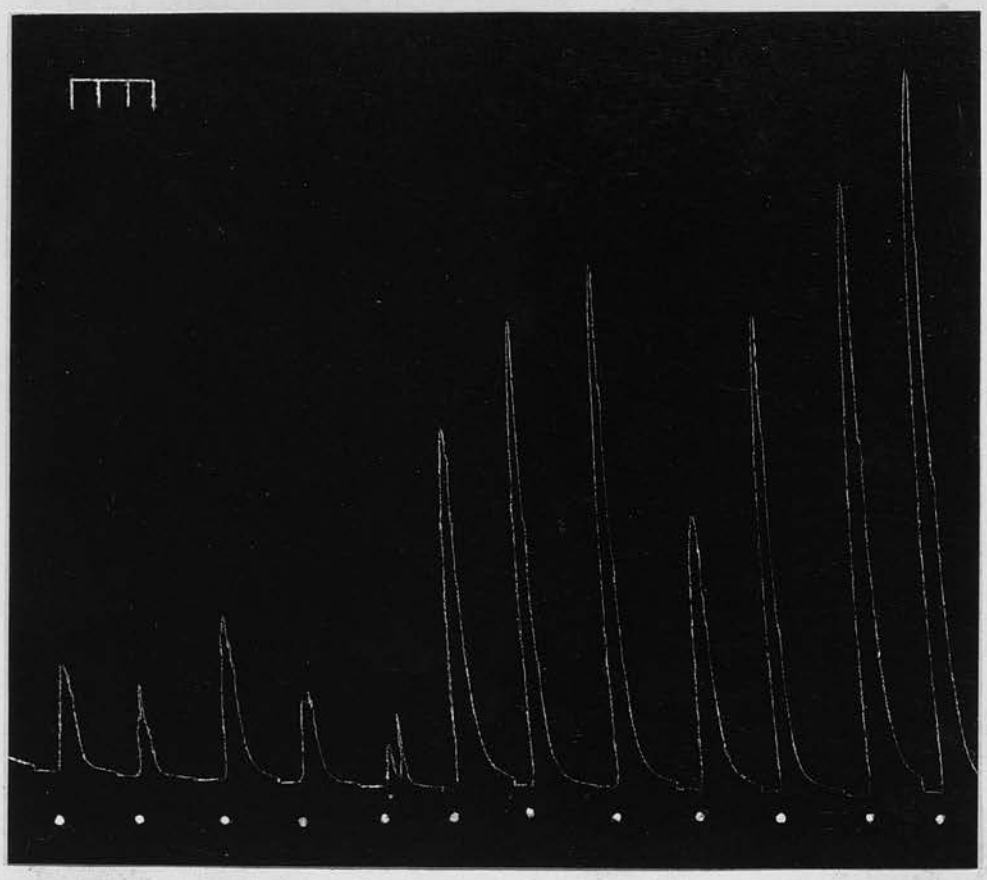


Responses to 5-hydroxytryptamine (HT 5 and 10 ng.), tryptamine (T, 1 and 2 µg.) and acetylcholine (Ach. 100 and 200 ng.) before, during and after maintenance of 5-hydroxytryptamine (1.25×10^{-9}) in the bath. This shows the synergism of all the three drugs with the small background dose of 5-hydroxytryptamine.

Time in min.

FIGURE V

Isolated rat uterus preparation



HT	Ach	HT	HT	Ach	HT	Ach	HT	HT	Ach	HT	Ach
10 ng	200 ng	5ng + Ach 100ng	10 ng	200 ng	20 ng	400 ng	10ng + Ach 200ng	20 ng	400 ng	40 ng	800 ng

Synergism between acetylcholine (Ach) and 5-hydroxy-tryptamine (HT). The responses to a combined dose of 5 ng of 5HT plus 100 ng. of Ach is not significantly greater than that of 10 ng. of 5HT or to 200 ng. of Ach. alone. Similarly, the response to a combined dose of 10 ng. of 5HT plus 200 ng. of Ach. is not significantly greater than that of 20 ng. of 5HT or to 400 ng. of Ach.

Time in min.

added as well, there was a response as great as that to 4 ng. 5-hydroxytryptamine previously. It was found that this effect was shown only by the purely stimulant compounds over a limited range of concentrations - because a fivefold increase was usually adequate for the transition from no effect at all to stimulation of the preparation. It was further possible to produce similar results with 5-hydroxytryptamine itself (Fig. IV) or with acetylcholine (5×10^{-7}).

Further evidence that the effect of this low concentration of acetylcholine on 5-hydroxytryptamine and tryptamine was merely an addition, was obtained by the following experiment. A contraction was obtained with a dose of 5-hydroxytryptamine (10 ng. in a 2 ml. bath) and a comparable contraction with acetylcholine (200 ng.). When half the dose (5 ng.) of 5-hydroxytryptamine was added at the same time at half the dose (100 ng.) of acetylcholine, the contraction produced was slightly, but not significantly, bigger than those obtained previously (Fig. V). This experiment was repeated, using twice the doses, with the same result, and also with tryptamine (1 μg. and 2 μg.) in place of 5-hydroxytryptamine.

TABLE 2

Antagonistic activity on the rat uterus using 5-hydroxytryptamine and tryptamine at 30°C

DOSE RATIO EXPERIMENTS

Compound No.	DRUG	Conc. (M)	DOSE RATIOS FOR: 5-Hydroxy-tryptamine	Tryptamine	Acetyl-choline
34	5-Benzyloxy-N-dipropyl-tryptamine	5.2×10^{-7}	4	2	1
		13	20	27	1
			4	8	1
		26	20	40	1
35	5-Benzyloxy-N-diisopropyl-tryptamine	6.5	4	2	1
			20	20	2
37	5- Benzyloxy-N-Morpholine-tryptamine	13	4	2	1
		26	4	4	1.5
38	5-Benzyloxy-α-methyl-tryptamine	7.8	5	8	1.5
			4	3	1
25	5-Hydroxy-N-dibutyl-tryptamine	30	10	5	0.3^{+}
			20	10	0.6^{+}
		76	20	25	0.5^{+}
3	N-Dimethyl-tryptamine	10	4	2.5	1
			3.5	2	1
		30	7	2.5	1
		100	5.5	1.5	1
			10	4	1
16	2-Methyl-N-dimethyl-tryptamine	100	1.5	-	1
			1.5	$<0.5^{+}$	$<0.5^{+}$
			1.0	0.25^{+}	0.25^{+}

$^{+}$ Indicates potentiation which was more marked at the end of 1 hr. After washing, this effect passed off much more quickly than did the antagonism to 5-hydroxytryptamine.

- Signifies not tested

(All the antagonists were kept in the bath for 1 hr.)

(ii) Antagonism of 5-hydroxytryptamine and tryptamine: This was only shown by those compounds listed in Tables II, III and IV, which summarise the results of quantitative experiments on the antagonistic properties of the compounds on this preparation. The antagonism took about 1 hr. to reach a maximum, and the same time to wear off. Gaddum, Hameed, Hathway and Stephens (1955) obtained drug ratios of 0.66 for 5-benzyloxygramine (41) and 0.027 for N-dimethyltryptamine (3)(3 calculated on a molar basis) which agree quite well with these results.

In all the experiments, whether the drug ratio or dose ratio, control responses to acetylcholine were not suppressed. 5-Hydroxy-N-dibutyltryptamine (25) and 2-methyl N-dimethyltryptamine (16) actually left the tissue more sensitive to the effects of acetylcholine. It will be seen from Table III that, when the antagonists were present in high concentrations, the responses to 5-hydroxytryptamine did not increase as the concentration of 5-hydroxytryptamine was increased, and this gave a very high and unreliable value for drug ratio. This situation has been described by Gaddum, Hameed, Hathway and Stephens (1955) as an "unsurmountable block". High concentrations of some of the compounds

TABLE 3

Antagonistic activity on the rat uterus using 5-hydroxytryptamine at 30°C

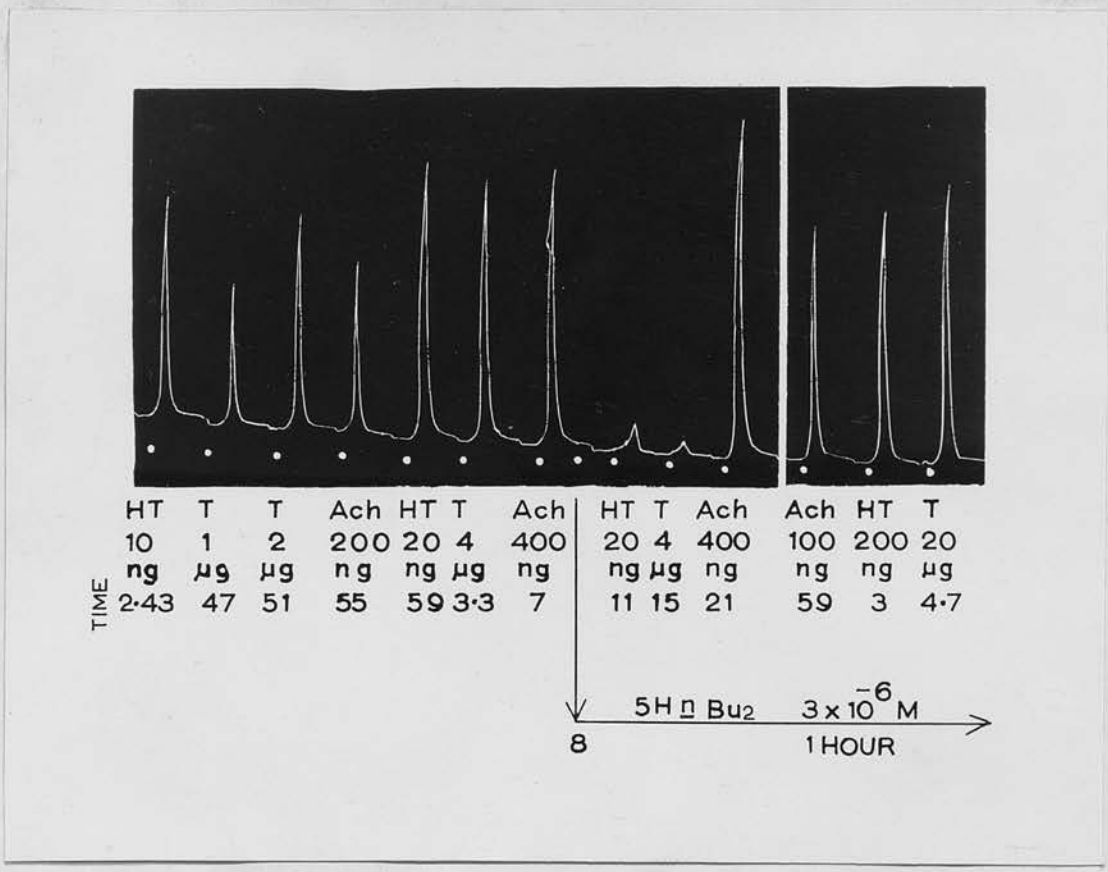
DRUG RATIO EXPERIMENTS

Compound No.	DRUG	Conc. (M)	Drug ratio (Mean±S.E.)	No. of Expts
41	5-Benzyloxygramine	9.0×10^{-7}	0.77	1
		18.0	0.56	1
		72.0*	6.4	1
32	5-Benzyloxy-N-dimethyltryptamine	90.0	0.066 ± 0.008	4
33	5-Benzyloxy-N-diethyltryptamine	7	0.074	1
		21	0.093	1
		55*	0.47, 9.25	2
34	5-Benzyloxy-N-dipropyltryptamine	5.2	0.095	1
		13.0	0.107 ± 0.064	2
		26.0	0.095	1
35	5-Benzyloxy-N-diisopropyltryptamine	6.5	0.076 ± 0	2
		13.0*	0.57, 0.95	2
37	5-Benzyloxy-N-morpholinotryptamine	13.0	0.06	1
		26.0	0.03	1
38	5-Benzyloxy-α-methyltryptamine	7.8	0.16	1
		32.0	0.27	1
		78	0.12	1
25	5-Hydroxy-N-dibutyl tryptamine	30	0.10 ± 0.035	2
		75	0.054	1
3	N-Dimethyltryptamine	99.0	0.042 ± 0.014	4

*Indicates that at the high concentration, the block became unsurmountable as previously shown by Gaddum, Hameed, Hathway and Stephens (1955) using 5-benzyloxygramine.

Figure VI

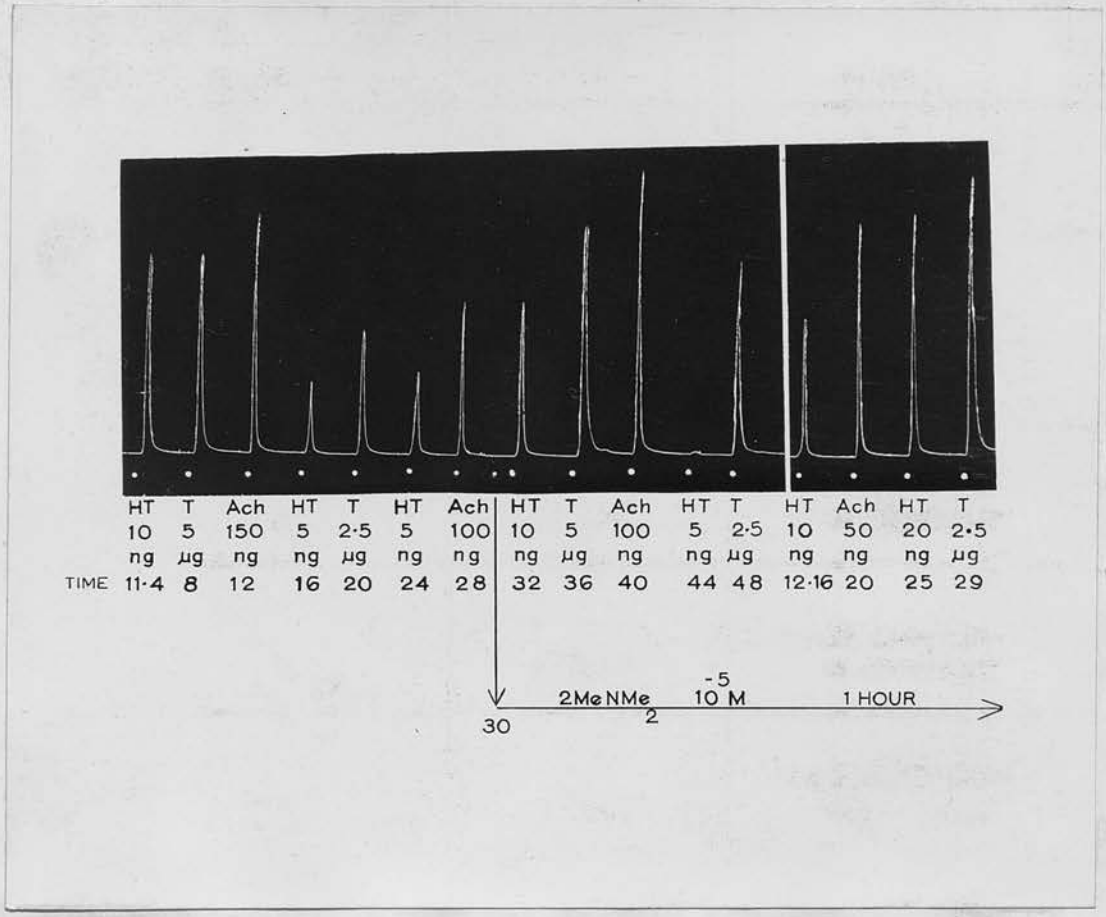
Isolated rat uterus preparation



Antagonism of 5-hydroxytryptamine (HT) and tryptamine (T) by 5-hydroxy-N-dibutyltryptamine (5HnBu$_2$). Responses are shown to HT (10, 20 and 200 ng.), T (1, 2, 4 and 20 µg.) and acetylcholine (Ach. 100, 200 and 400 ng.) before and after the action of the analogue (3×10^{-6}M) for 1 hr. At the end of the experiment, dose ratios for HT was 10, for T it was 5 and for Ach. it was 0.3.

Figure VIII

Isolated rat uterus preparation



Effect of a low concentration of 2-Methyl-N-dimethyl-tryptamine ($2MeNMe_2$). Responses are shown to 5-hydroxytryptamine (HT 5, 10 and 20 ng.); tryptamine (T 2.5 and 5 µg.) and acetyl-choline (Ach. 50, 100 and 150 ng.) before and after the action of the analogue (10^{-5} M) for 1 hr. At the end of 1 hr. the dose ratio for HT was 1.5; for T it was 0.5 and for Ach. it was 0.5

TABLE 4

Antagonistic activity of the feeble antagonists on the rat uterus using 5-hydroxytryptamine at 30°C

DOSE RATIO EXPERIMENTS

Compound No.	DRUG	Conc. (M)	DOSE RATIOS FOR:	
			5-hydroxytryptamine	Acetylcholine
4	N-diethyl-tryptamine	40×10^{-7}	4	1
16	2-Methyl-N-dimethyl-tryptamine	100	1.5 1.5 1.0	1 <0.5+ 0.5+
13	N-Methyl-tryptamine	125	3	1
9	N-Piperidine tryptamine	150-	2	1
10	N-Morpholino-tryptamine	200-	2	1
12	α-Ethyl-tryptamine	420	4	1

+ Indicates potentiation.
All the compounds were allowed to act for 60 mins. except the one marked -, which was allowed to act for 30 min. only.

producing such block also depressed the responses to acetylcholine.

Table II shows the dose ratios for 5-hydroxytryptamine and acetylcholine obtained with a number of antagonists. With one exception, the antagonism of tryptamine was about the same as that of 5-hydroxytryptamine. The exception 2-methyl-N-dimethyltryptamine (16) appeared to potentiate responses to both tryptamine and acetylcholine to some extent. This potentiation was greatest at the end of about one hour, (Fig.VIII).

It is convenient to classify the compounds into

(a) antagonists comparable with lysergic acid diethylamide (which has a drug ratio of 37 on a molar basis;

(b) moderate antagonists, such as 5-benzyloxygramine (41); 5-benzyloxy N-dimethyltryptamine (32) and the compounds listed in Table III, which shows their drug ratios;

(c) feeble antagonists, such as compounds shown in Table IV.

These compounds had such weak activity that the dose ratio only was determined. The highest value of these was below the limit (about 5) at which it is possible to measure the drug ratio.

All the 5-benzyloxy-3-(2-dialkylaminoalkyl

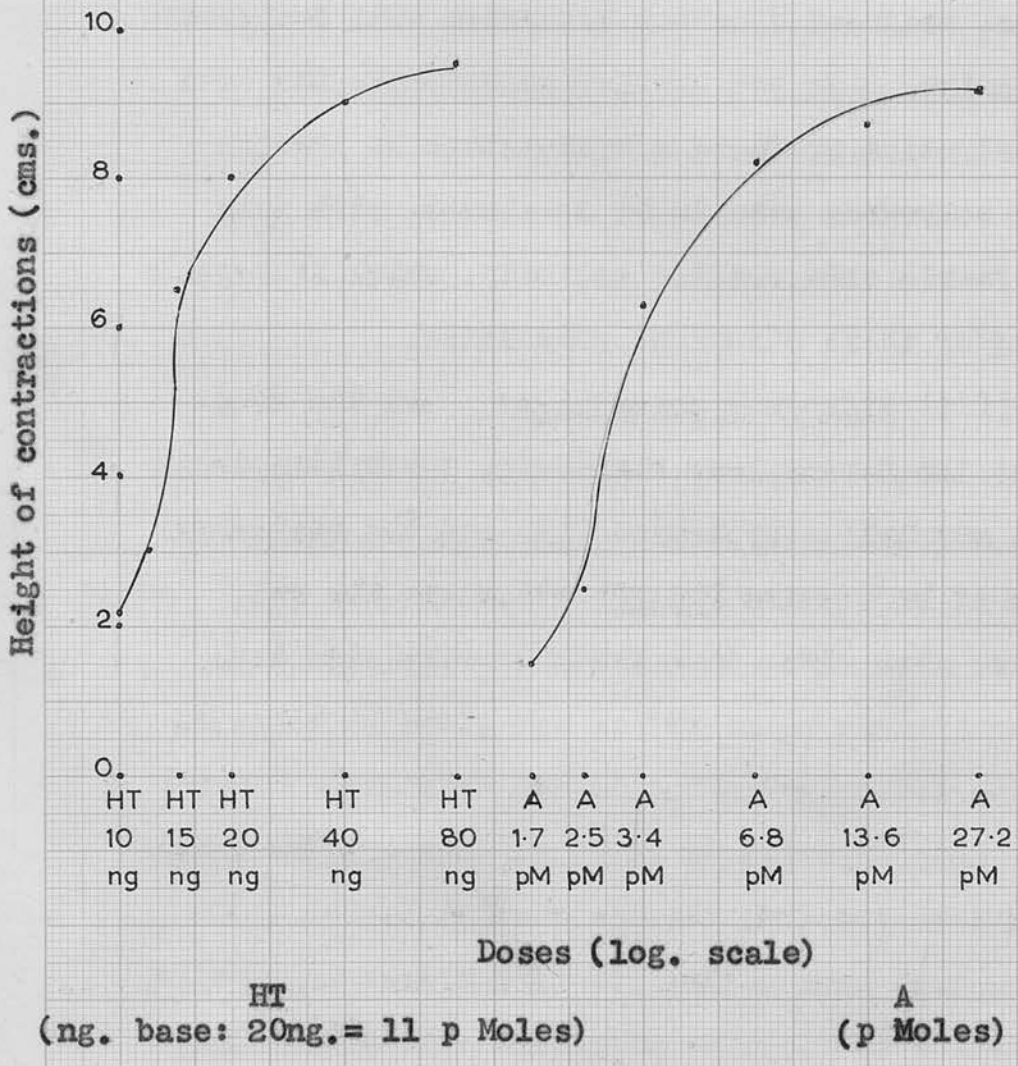
indoles seemed to have the same order of activity, although there was an increase up the series to the dipropyl compound (34), but none was as active as 5-benzyloxygramine (41). This was disappointing, because Gaddum, Hameed, Hathway and Stephens (1955) had shown that 5-benzyloxygramine (41) was quite a potent antagonist of 5-hydroxytryptamine, and it might be expected that 5-benzyloxy-N-dimethyltryptamine (32) would be more active because it contained the aminoethyl sidechain present in 5-hydroxytryptamine. The dibutyl compound (36) was not very soluble and in the highest concentration tested (about 6×10^{-5} M) did not have any effect. The corresponding 5-hydroxy compound (25) was the only 5-hydroxy compound which antagonised 5-hydroxytryptamine. In the N substituted tryptamine, it was only N-dimethyltryptamine (3) which had antagonistic activity. This was one-eighth as powerful as the corresponding 5-benzyloxy compound (32). The most active antagonist was 5-benzyloxy-α-methyltryptamine (38), but even this was less active than 5-benzyloxygramine (41).

N-Diethyltryptamine (4), when tested in one experiment (Table 4a) blocked 5-hydroxytryptamine, but the same concentration (4×10^{-6} M) on a different tissue caused synergism with 5-hydroxytryptamine,

FIGURE A

Rat uterus

Log. dose response curves to 5-hydroxytryptamine (HT) and 5-hydroxy-N-dipropyltryptamine (A).



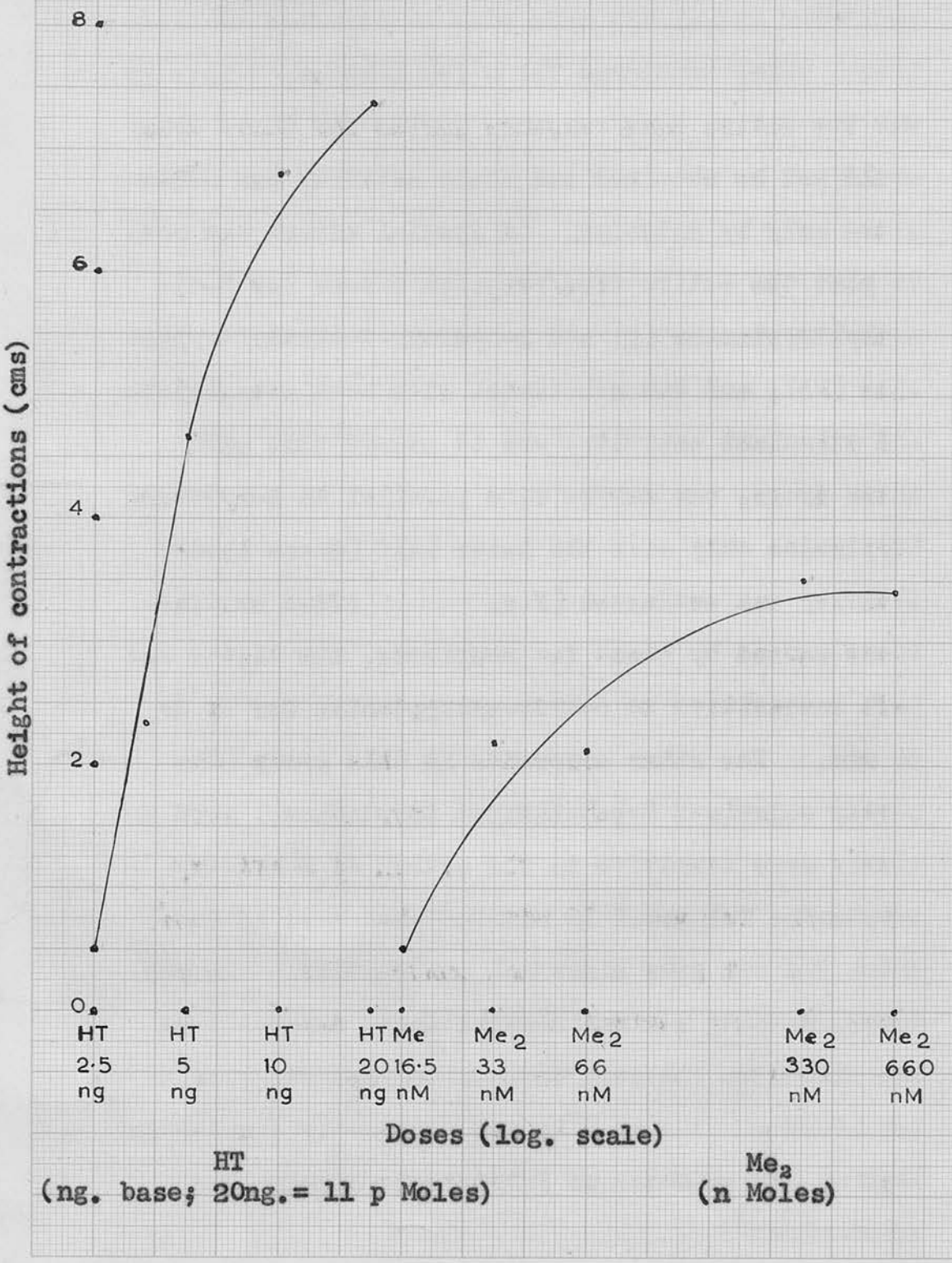
and in yet another experiment, in the same concentration, caused contraction of the preparation. Similarly, 1-methyl-α-methyltryptamine (19) in one experiment antagonised 5-hydroxytryptamine, giving a dose ratio of 4, but in a second experiment (in the same concentration of 3×10^{-6} M) on a different tissue, produced apparent synergism with 5-hydroxytryptamine. In yet another experiment, the same concentration produced a contraction by itself. These compounds seem to be substances with only very feeble antagonistic activity, which is usually masked by their stimulant properties.

(iii) Stimulant properties: All the drugs except the 5-benzyloxy compounds, α-ethyltryptamine (12), morpholinotryptamine (10) and the quaternary salts of bufotenine (26,27) stimulate the rat uterus, when given in a suitable concentration. The equipotent molar ratios are given in Table 1. As with 5-hydroxytryptamine and tryptamine, there was a latent period of 15-20 sec. before the muscle started to respond, the contraction lasted about 30 sec. and then passed off. 5-hydroxytryptamine, tryptamine and all the stimulant analogues, except the N-substituted tryptamines and 5-hydroxy-dibutyltryptamine (25) were easily washed out and the preparation returned to normal rapidly. The

FIGURE B

Rat uterus

Log. dose response curves to 5-hydroxytryptamine (HT) and N-dimethyltryptamine (Me_2).



exceptions required more washing before the tissue recovered and always gave rise to repeated contractions, which continued for a few minutes. This is why the entire dose/response curves for these drugs could not be obtained and their activity was determined only by matching. A special effort was made to plot the entire dose/response curves for N-dimethyltryptamine (3) and 5-hydroxy-N-dibutyltryptamine (25), and two substances with both antagonistic and stimulant activity, and it seemed that with these drugs, the curves were parallel to 5-hydroxytryptamine only over the lower half (where their activity was estimated (Fig. B). After contractions caused by these two compounds, the tissue was left insensitive to 5-hydroxytryptamine for up to 30 min. The other compounds in this group (the purely stimulant N-substituted tryptamines) left the tissue more sensitive to the action of 5-hydroxytryptamine for about 10 minutes when used in doses which did not give a maximal contraction. Larger doses, causing maximal contractions, desensitised the tissue to the test dose of 5-hydroxytryptamine for 10-15 minutes in a manner apparently similar to that of larger doses of 5-hydroxytryptamine and tryptamine.

The remaining compounds (the 5-hydroxy-.

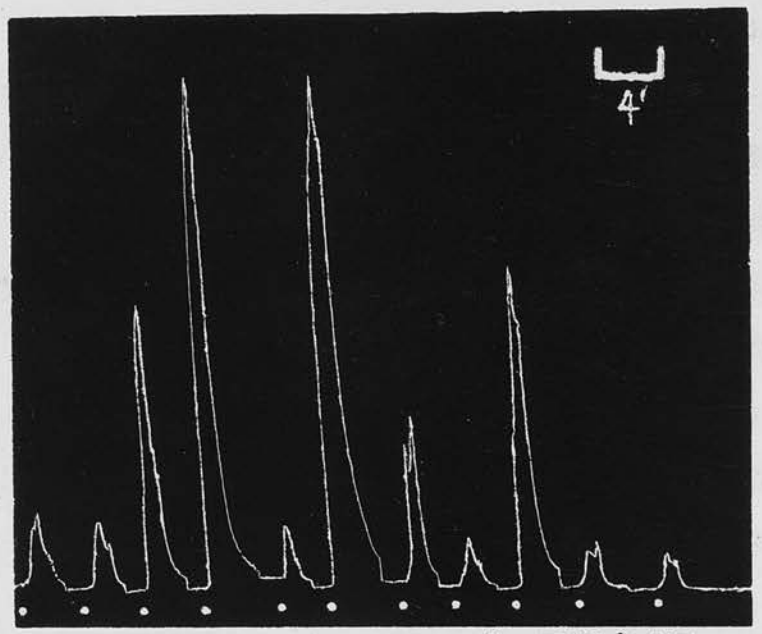
5-methyl-, 5-methoxy- and α-methyl derivatives of tryptamine) caused contractions apparently the same as those produced by 5-hydroxytryptamine and tryptamine, and were easily washed out. Their dose/response curves were parallel to that of 5-hydroxytryptamine and these drugs only affected the response to the test dose of 5-hydroxytryptamine, when high doses were used. In these circumstances, they reduced the response slightly.

The most active compound was 5-hydroxy-α-methyltryptamine (20) and the next most active were 5-methoxy-α-methyltryptamine (31) and bufotenine (21). The activity in the series of 5-hydroxy-N-dialkyltryptamines declined as the length of the alkyl group was increased, whereas, in contrast, the activity rose slightly from the dimethyl to the dipropyl compound in the series of simple N-dialkyltryptamines. The 2-methyl were almost as active as the simple N-dialkyl tryptamines except the dimethyl compound, 2-methyldimethyltryptamine (16), which was less than one-twentieth as active as N-dimethyltryptamine (3). The three N-monoalkyl analogues had activity much lower than the corresponding N-dialkyl derivatives.

The effects of the size of the alkyl group on activity is shown in Fig. G & H. The 5-methyl group,

Figure IX

Isolated rat uterus preparation



Stimulant activity of 5-hydroxytryptamine (HT 5, 10 and 20 ng.) and 5-hydroxy-N-di*iso*propyltryptamine (IPr_2HT 0.2, 0.4 and 0.8 n. Mole). A 5 ng. test dose of 5-HT is applied before every application of the analogue, to make sure that the sensitivity of the uterus is uniform.

10 ng. of HT = 5.5 p. Moles.

and to a much greater extent, the 5-benzyloxy group and the ethyl group at the α-position, reduced the stimulant activity. The quaternary salts were inactive, as were also, although these are not included in the table (I), the glyoxylamides, 3-(5-benzyloxy)-indolylglyoxyldimethylamide and 3-(2-methyl)-indolylglyoxyldimethylamide, which were intermediates in the synthesis of the tryptamines and hydroxytryptamines. 5-Hydroxy-α-methyltryptamine (20) was half as active as 5-hydroxytryptamine; 5-methyl- α-methyltryptamine (31) was 1/8th as active as 5-methyltryptamine (30), 5-methoxy-α-methyltryptamine (29) was twice as active as 5-methoxytryptamine (28) and α-methyltryptamine (11) 1/4 as active as tryptamine.

(iv) Production of repeated contraction: 5-hydroxytryptamine and tryptamine, when applied to the tissue for 5-10 min. in a concentration somewhat higher than the one causing maximum contraction, caused repeated contractions. All the compounds, except the 5-benzyloxy derivatives also did this when added to the bath in a high enough concentration. Some of the N-substituted tryptamines (see p. 82), produced repeated contractions which persisted for some time, even when applied in moderate doses and after repeated washing of the

preparation. After such treatment, the tissue was more sensitive to the action of 5-hydroxytryptamine for a few responses, but, when repeated contractions were produced by the compounds including 5-hydroxytryptamine and tryptamine, the tissue was less sensitive to the test dose of 5-hydroxytryptamine for a few minutes.

(v) Profound depression: In still higher concentrations, the preparation was quiet and insensitive to 5-hydroxytryptamine. The responses to acetylcholine were not always affected, but were blocked by raising the concentration of the drug still further.

2. EFFECTS ON THE RAT FUNDUS STRIP

The highest concentrations tested on this preparation were not usually greater than those which produced a maximum contraction (varying with the stimulant activity of the compounds, usually around 10^{-5} M), because on the occasions when the drugs were tested in concentrations above this, they did not give rise to repeated contractions or profound depression, such as was observed in the rat uterus. The actions of these compounds can again be divided into:

(i) Modification of responses to 5-hydroxytryptamine.

(ii) Stimulant action.

TABLE 5

Antagonistic activity of bromo-lysergic acid on the rat fundus strip at 37°C to 5-hydroxytryptamine and other indoles

DOSE RATIO EXPERIMENTS FOR 1 HR.

DRUG	Conc. (M)	DOSE RATIOS FOR:					
		5-HT	Trypt-amine	5-Methyl-tryptamine	Me_2N	nPr_2N	Acetyl-choline
Bromo-Lysergic-acid di-ethylamide	0.5×10^{-7}	1.5	1	-	-	-	1
	1.2	40	4	-	-	-	1
		50	7	-	40	-	1
		15	-	-	-	15	1
		5	2	-	>10	-	1
		10	5	-	-	10	1
		75	10	15	-	-	1
	2.5	20	7	-	-	-	1
		60	8	8	-	-	1
	3.7	40	10	-	-	40	1
		25	6	-	53	-	1
		400	20	20	-	-	2
	7.5	100	10	-	-	-	1
	25.0	130	>10	-	-	-	1
		>200	6	-	-	-	1

5-HT = 5-hydroxytryptamine

Me_2N = N-dimethyl-tryptamine (3)

nPr_2N = N-dipropyl-tryptamine (5)

TABLE 6

Antagonistic activity of indoles on the rat fundus at 37°C to 5-hydroxytryptamine and tryptamine

DOSE RATIO EXPERIMENTS

Compound No.	DRUG	Conc. (M)	DOSE RATIOS FOR:		
			5-Hydroxy-tryptamine	Tryptamine	Acetyl-choline
41	5-Benzyloxy-gramine	18×10^{-7}	2.5	1	1
		36	5	2	1
			5	2	1
		130	15	2	1
		180	27	8	2
		360	100	20	1
32	5-Benzyloxy-N-dimethyl-tryptamine	6	10	2	<1
			10	2	<1
3	N-dimethyl-tryptamine	1	2	1	1
		3.3	10	2	1
			10	<1	1
			10	1.5	1
16	2-Methyl-N-dimethyl-tryptamine	20	10	0.5 - 1*	1
			5	0.5 - 1*	1
42	1-Benzyl-2-methyl-5-methoxy-tryptamine	3.1	1	1	1
		31.0	2	1	1
		78.0	4	1	1
	Bromo-lysergic acid di-ethylamide+	1.2	40	4	1
		2.5	20	7	1
		3.7	40	10	1
		25.0	>200	6	1

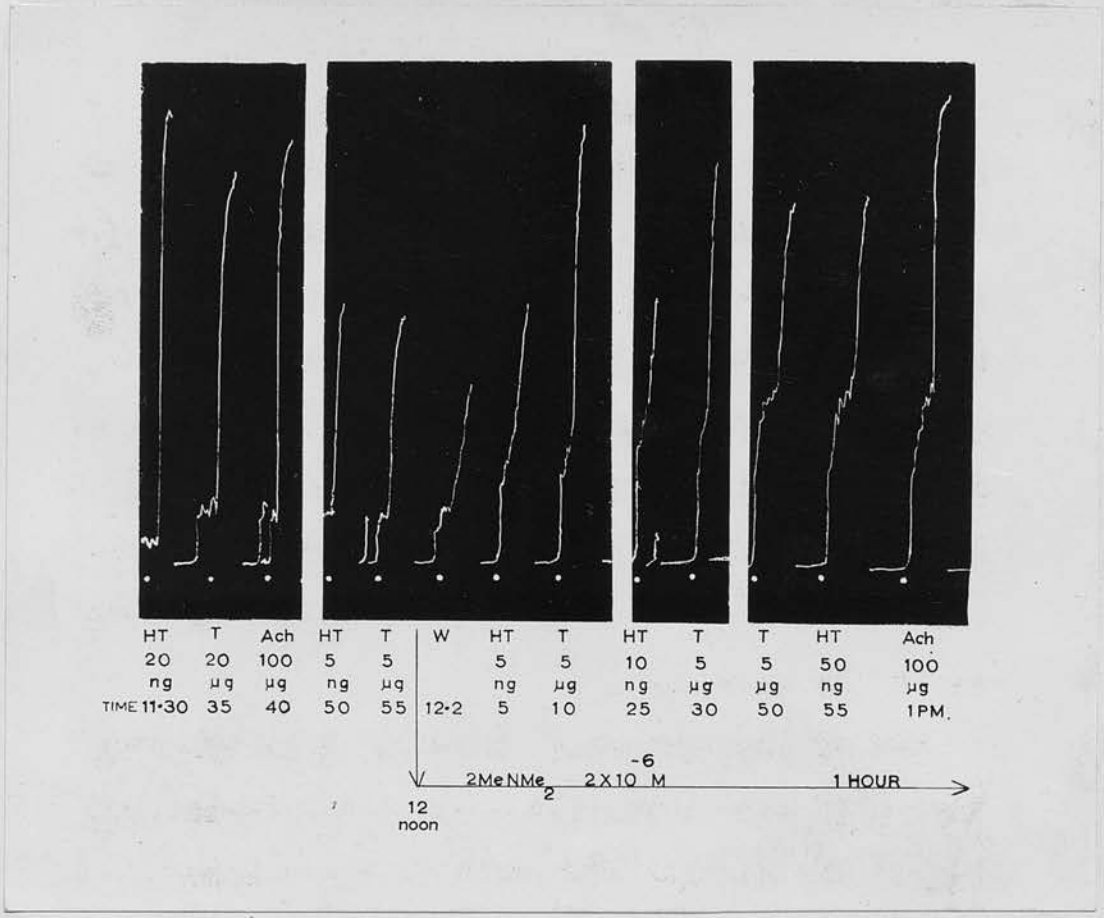
+ Results with bromo-lysergic acid diethylamide have been included from Table 5 for comparison.

The antagonistic effect required 30-40 mins. to develop completely, and this period was allowed for the action of 1-Benzyl-2-methyl-5-methoxytryptamine, but in experiments with other compounds, they were allowed to act for 60 mins.

* Means potentiation, which was much marked at the beginning of the experiment.

Figure X

Rat fundus strip preparation



Effect of a low concentration ($2x10^{-6}$M) of 2-methyl-N-dimethyltryptamine ($2MeNMe_2$). Responses are shown to 5-hydroxy-tryptamine (HT 5, 10, 20 and 50 ng.). Tryptamine (T, 5 and 20 µg.) and acetylcholine (Ach. 100 µg.) before and after the action of the analogue. At 12 noon, when the analogue was first maintained in the bath, the base line rose by about 3 cms. and the tissue was given an additional wash (W), and stretched. The base line tended to stay higher during the rest of the experiment. Dose ratios at the end of 1 hr. were 10 for HT; about 1 for T and Ach. The T responses were significantly potentiated in the early part of the experiment. Hyoscine $2.3x10^{-7}$M maintained in Tyrode solution.

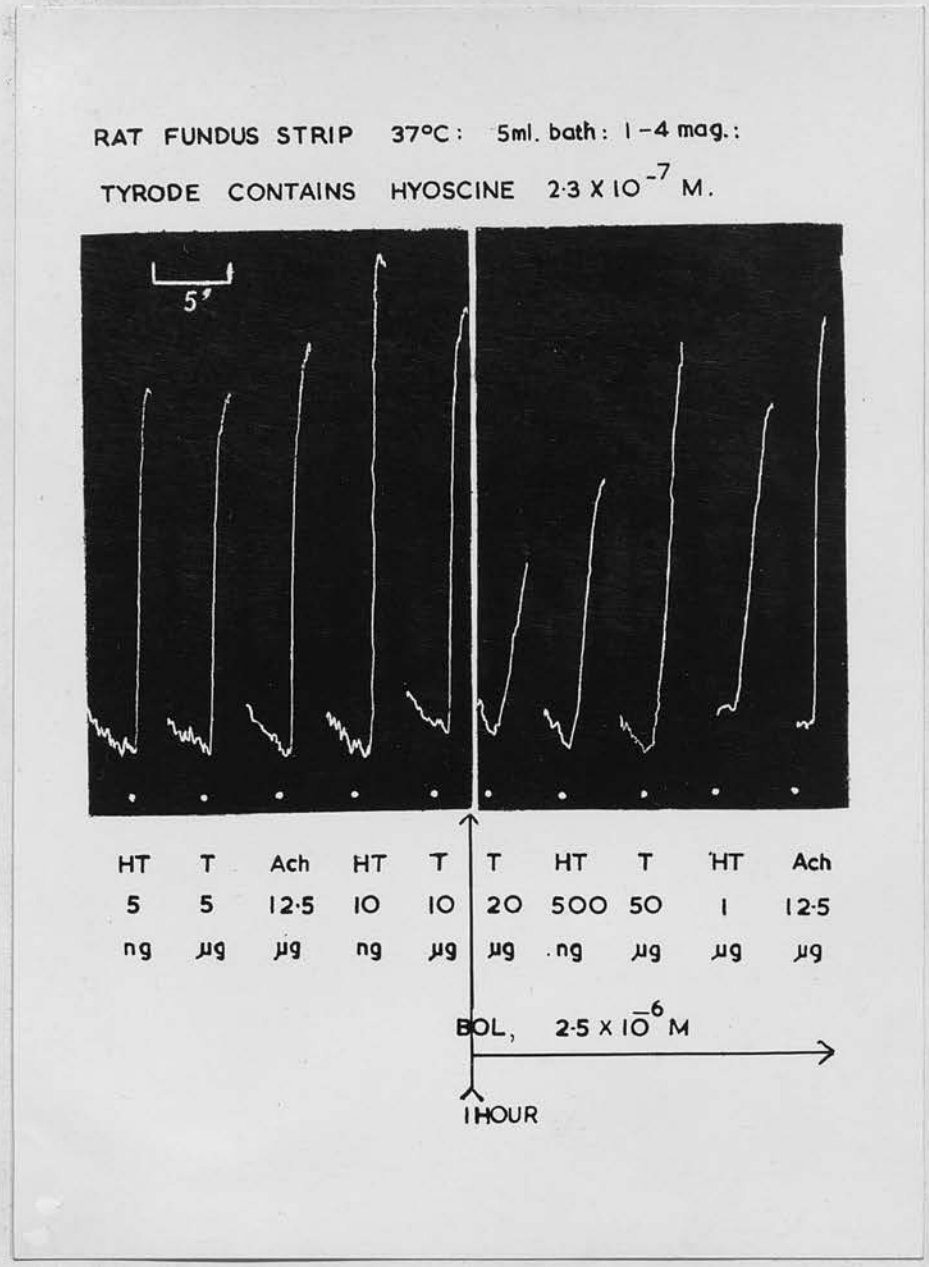
(iii) Depression of 5-hydroxytryptamine response.

(i) Modification of the responses to 5-hydroxytryptamine and Tryptamine. To see if there was any synergism with 5-hydroxytryptamine or tryptamine at low concentrations, such as was observed on the rat uterus, the drugs were added to the tyrode solution so that the preparation was continuously in the presence of a low concentration (10^{-8} M) of the compounds. None of them showed any synergism with 5-hydroxytryptamine, even when allowed to act for 60 min. in a concentration high enough to cause a rise of the base line.

Two of the compounds, however, 2-methyl-N-dimethyl (16) and 2-methyl-N-diethyl-tryptamine (17) enhanced the initial responses to tryptamine.

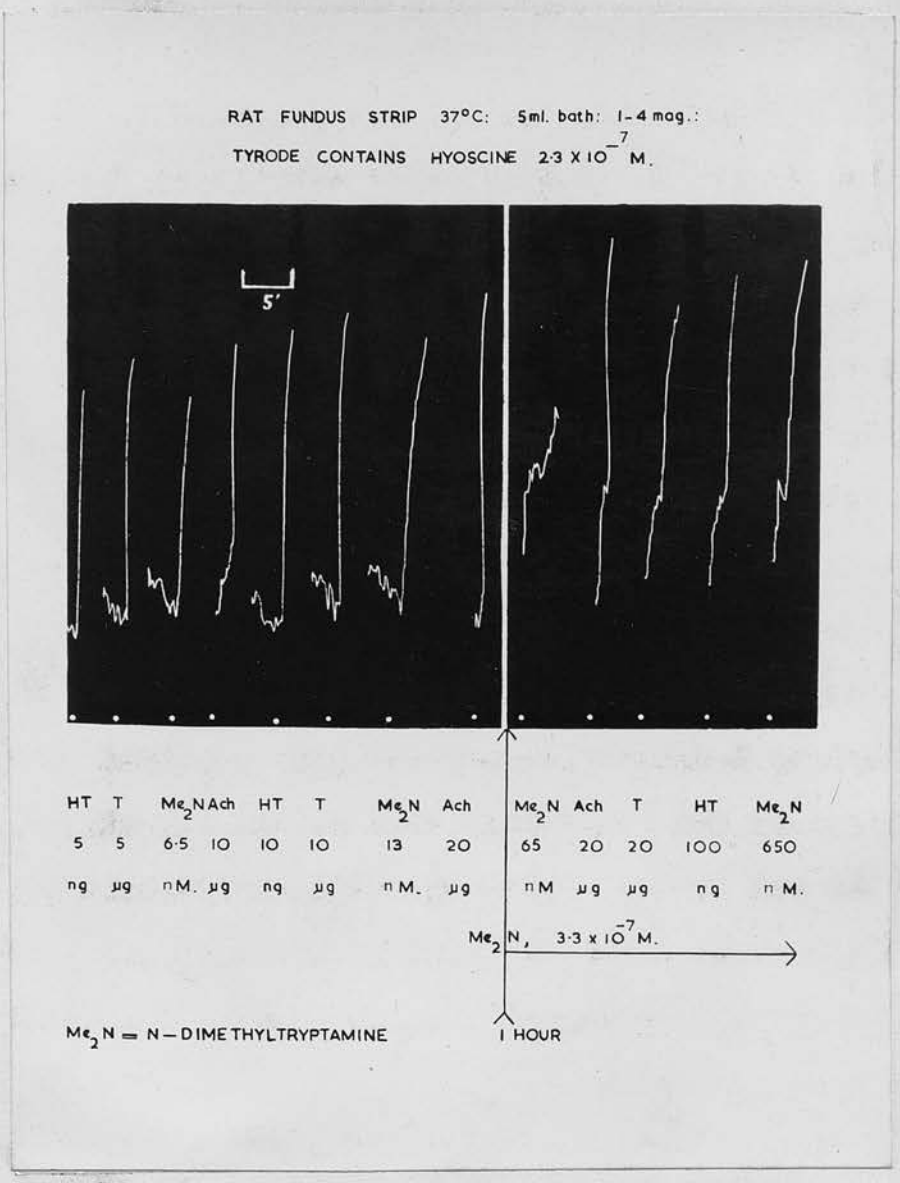
Three of the compounds, N-dimethyl-, 2-methyl-N-dimethyl and 5-benzyloxy-N-dimethyl tryptamines (3, 16, 32), antagonised 5-hydroxytryptamine, but did not antagonise tryptamine to quite the same extent. The dose ratios of these and of 5-benzyloxygramine (41) which also blocked 5-hydroxytryptamine to a greater extent than tryptamine, are given in Table 5&6. The 2-methyl-N-dimethyltryptamine (16) enhanced the responses to tryptamine at the same concentration as it antagonised 5-hydroxytryptamine. This effect was much more noticeable in the

Figure XI



Effect of bromolysergic acid diethylamide (BOL 2.5×10^{-6} M). Responses are shown to 5-hydroxytryptamine (HT 5, 10 and 500 ng. and 1 µg.). Tryptamine (T5, 10, 20 and 50 µg.) and acetylcholine Ach. 12.5 µg.) before and after the action of BOL for 1 hr. Note that the antagonism of HT is 25 times that of T, while Ach responses are unaffected. Hyoscine 2.3×10^{-7} M maintained in Tyrode solution.

Figure XII



Effect of a low (3.3×10^{-7} M) concentration of N-dimethyltryptamine. Responses are shown to 5-hydroxytryptamine (HT 5, 10 and 100 ng.); tryptamine (T, 5, 10 and 20 µg.); analogue (6.5, 13, 65 and 650 n. Moles) and acetylcholine (Ach. 10 and 20 µg.) before and after the action of a low concentration of the analogue. Note that the base line has gone up by about 3 cms. after the analogue was maintained in the bath. The dose ratios at the end of 1 hr were 10 for HT., about 2 for T, 50 for the analogue and 1 for Ach. Hyoscine 2.3×10^{-7}M maintained in Tyrode solution.

beginning of the experiment than at the end (Fig.X). N-Dimethyltryptamine (3) antagonised 5-hydroxy-tryptamine but did not have any significant effect against tryptamine. It was not possible to test higher concentrations of these substances, because these drugs themselves caused contractions (Fig.XIII). The antagonism reached its maximum at about 1 hr. and the recovery process also took about the same time.

On this preparation 5-benzyloxygramine (41) was a less potent antagonist than 5-benzyloxy-N-dimethyl-tryptamine (32) and even less than N-dimethyl-tryptamine (3). The antagonistic effects of 5-benzyloxy-N-dimethyltryptamine (32) appeared to be greater on the rat fundus than on the rat uterus. On the rat fundus strip, N-dimethyltryptamine (3) and 5-benzyloxy-N-dimethyltryptamine (32) were about equi-active, whereas on the rat uterus, the latter was more powerful.

This preparation seems to be very sensitive to the stimulant properties of these compounds. Antagonistic activity was confined to the three compounds which contained the dimethylamino group.

(ii) Stimulant action. All the compounds, except N-morpholinotryptamine (10), all the quaternary salts (26, 27, 39, 40), 5-benzyloxy-N-dibutyl and

RAT FUNDUS STRIP

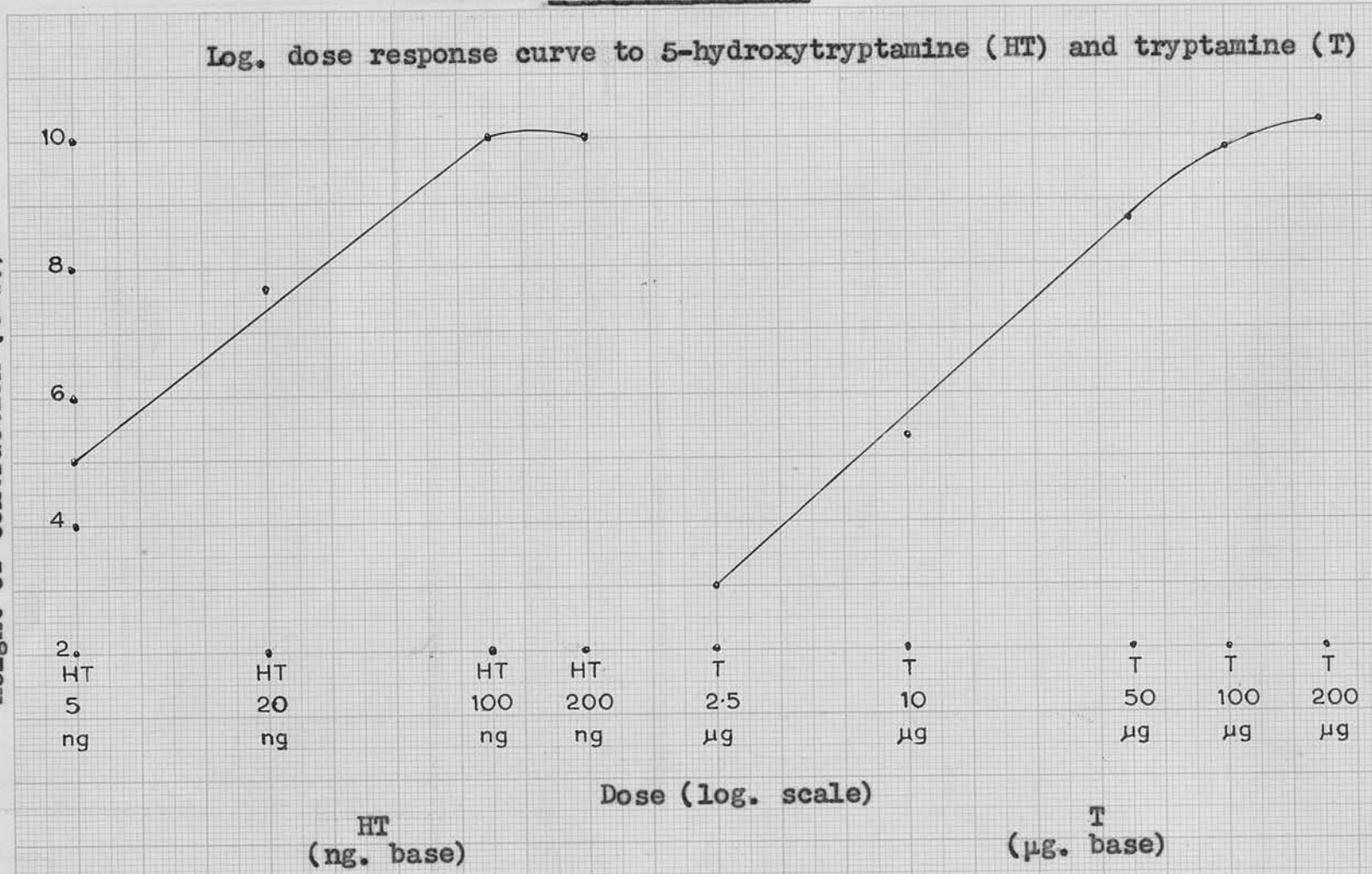
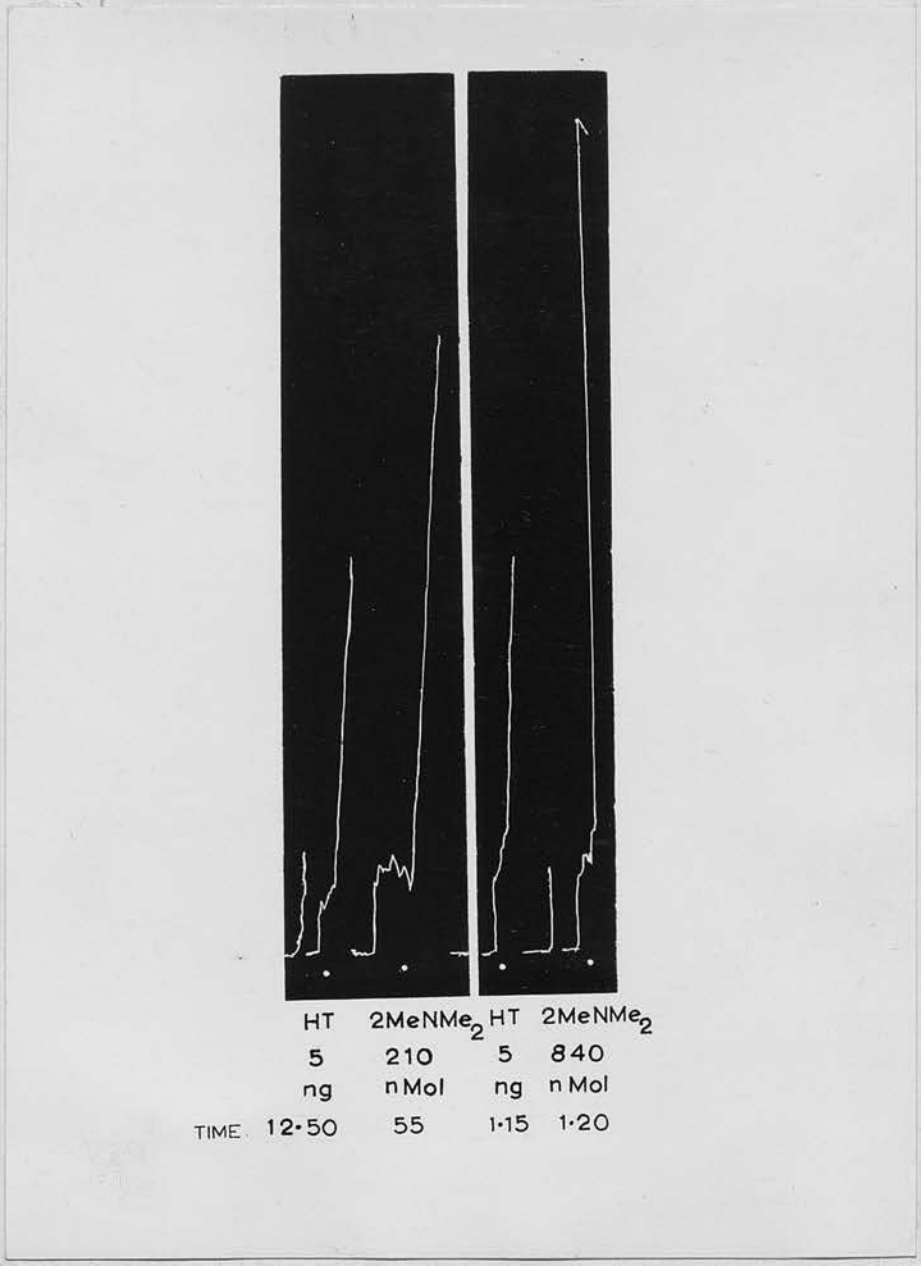


FIGURE C

Figure XIII

Rat fundus strip preparation

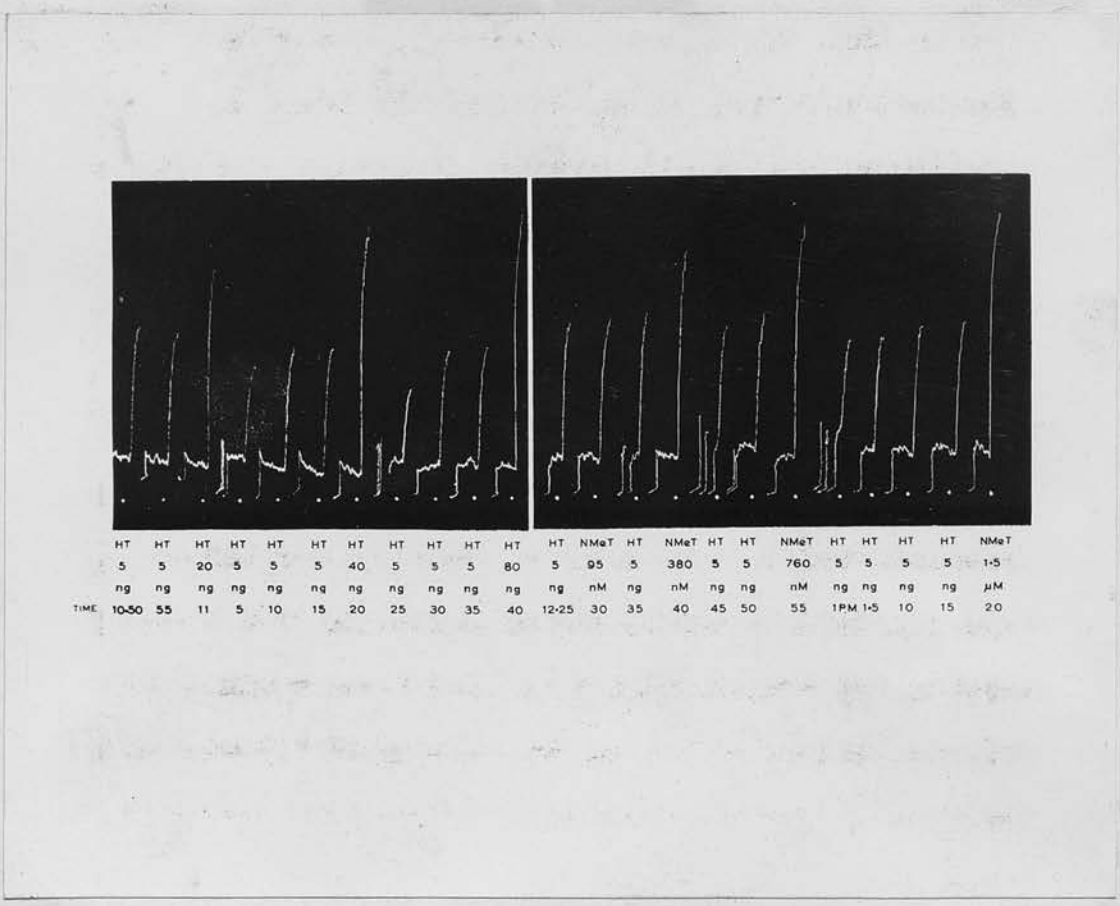


Stimulant activity of 2-methyl-N-dimethyltryptamine ($2MeNMe_2$). Responses are shown 5o 5-hydroxytryptamine (HT, 5 ng.) and the analogue (210 and 840 n. Moles). Hyoscine 2.3×10^{-7} M maintained in Tyrode solution.

HT 5 ng. = 2.8 p. Moles.

Figure XIV

Rat fundus strip preparation



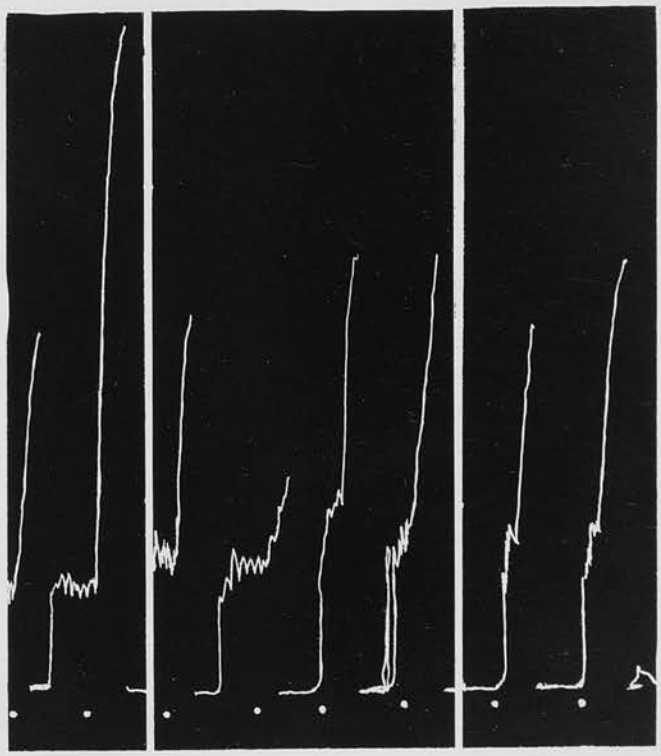
Stimulant activity of 5-hydroxytryptamine (HT) and N-methyltryptamine (NMeT). Responses are shown to HT (5, 20, 40 and 80 ng.) and to the analogue (95, 380 and 760 n. Moles and 0.5 µ. Moles). Note that the 5 ng. test dose of HT is applied in between the high concentrations of HT and all the applications of the analogue to see that the sensitivity of the preparation is uniform. After higher concentrations of HT and the different applications of the analogue there is a tendency for the base line to go up and so additional period of stretch was applied (multiple verticle lines). Hyoscine 2.3 x 10^{-7}M present in Tyrode solution.

N-morpholinotryptamines (36, 37) and the intermediate glyoxylamides (mentioned on p.84) stimulated the preparation. The compounds listed as antagonists in Table 6 also showed this effect at concentrations greater than those which produced antagonism. The stimulant activity is summarised in Table 1. 5-hydroxytryptamine, tryptamine, 5-hydroxy-α-methyltryptamine (20), 5-methoxytryptamine (28) and 5-methyltryptamine (30) caused contractions which reached a maximum within 90-120 sec. and which passed off after washing and stretching for about one minute. On the other hand, those 5-benzyloxy compounds which were active, α-methyltryptamine (11) 3-(2-dipropyl-N-dipropyltryptamine (5) and 1-methyl-α-methyltryptamine (19) required 2 to 3 mins. for the completion of the contractions, and 30-45 min. for stretching and recovery. Thus one assay of these long-acting drugs requires a whole day. The remaining compounds fall between these two extremes.

The dose/response curves of all the compounds (except those containing a 5-benzyloxy group) appeared to be parallel to those of 5-hydroxytryptamine and tryptamine. The 5-benzyloxy compounds did not give a maximal response, although at lower dose levels the curves were parallel to those of 5-hydroxytryptamine, and it was at these levels that the equipotent molar ratios were determined (Fig. D).

Figure XV

Rat fundus strip preparation



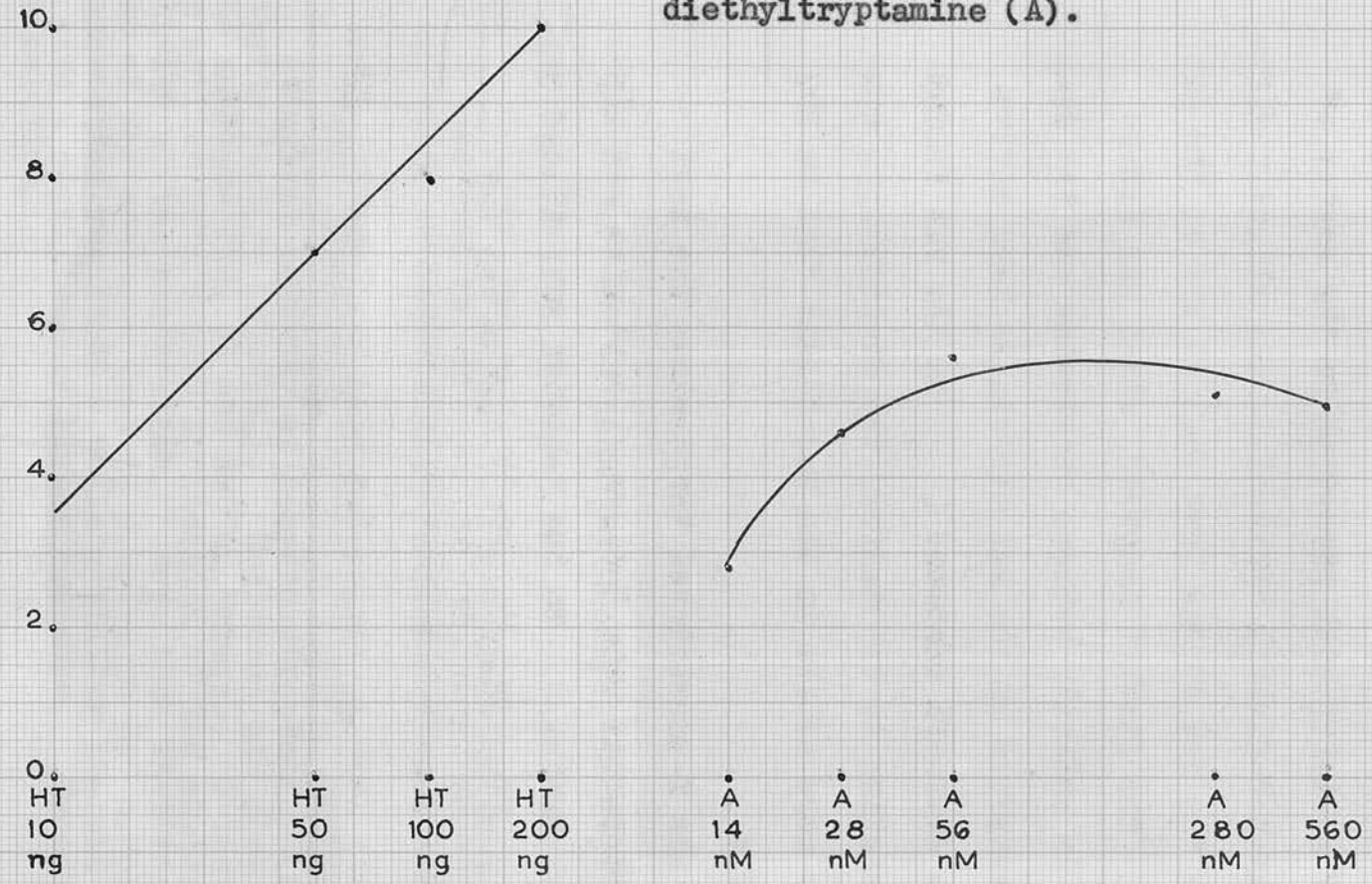
	HT	HT	HT	$5BzOEt_2$	HT	$5BzOEt_2$	HT	$5BzOEt_2$
	10	100	10	14	10	56	10	560
	ng	ng	ng	nM	ng	nM	ng	nM
TIME	11·20	25	40	45	50	55	12·15	20

Stimulant activity of 5-hydroxytryptamine (HT) and 5-benzyloxy-N-diethyltryptamine ($5BzOEt_2$). Responses are shown to HT (10, 100 ng.) and the analogue (14, 56 and 560 n. Moles). A test dose of 10 ng. of HT was applied before each application of the analogue to test for the sensitivity of the preparation. Note also that increasing the concentration of the analogue from 56 n.Moles to 560 n.Moles, no increase in the contraction resulted. While a similar 10-fold increase in the dose of HT gave a significantly higher response. Hyoscine 2.3×10^{-7} M in Tyrode solution.

FIGURE D

Rat fundus strip

Log. dose response curves to 5-hydroxytryptamine (HT) and 5-benzyloxy-N-diethyltryptamine (A).



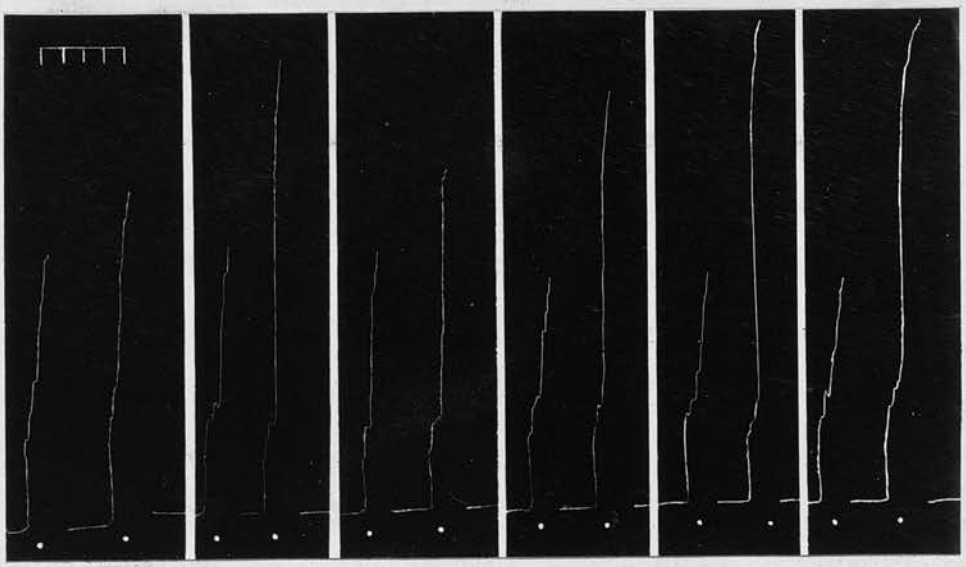
The most active compounds were 5-hydroxy-α-methyltryptamine (20) and 5-methoxy-α-methyltryptamine (29). The next most active were bufotenine and 5-hydroxy-N-di-isopropyltryptamine. The variation of activity with structure in the series of 5-hydroxy-2-N-dialkyl tryptamines was slightly different on the rat fundus from what it was on the rat uterus. The diethyl compound, which was intermediate in activity between bufotenine and the di-n-propyl compound on the rat uterus, is less active than the dipropyl compound (23) on the rat fundus. Also, relative to tryptamine, the di-isopropyl compound is more active on the rat fundus than on the rat uterus.

In the N-substituted tryptamines the most active compounds were N-dipropyltryptamine and alpha-methyltryptamine. In the 2-methyl substituted compounds, 2-methyl-N-dipropyltryptamine was most active and 2-methyl-N-dimethyltryptamine was only relatively feeble, suggesting that the 2-methyl group lowered the stimulant activity of N-dimethyltryptamine considerably. In the monoalkyl substituted tryptamines, the most active compound was the ethyl derivative. The effect of the size of the alkyl group on activity is shown in Fig. G and H.

It was noted that, whereas α-methyltryptamine

FIGURE XVI

Isolated rat fundus preparation



Stimulant action of 5-benzyloxy-N-dimethyltryptamine.

a. Responses to 10 ng. of 5-hydroxytryptamine (10) and 0.3 μ.M. of 5-benzyloxy-N-dimethyltryptamine (0.3) at time 12.28.
b. Responses to 10 ng. and 100 ng. of 5-hydroxytryptamine (10 and 100) at 14.05.
c. Responses to 10 ng. of 5-hydroxytryptamine (10) and a combined dose of 0.3 μ.M. of 5-benzyloxy-N-dimethyltryptamine plus 100 ng. of 5HT (0.3 + 100) at 14.20.
d. Responses to 10 ng. and 100 ng. of 5HT at 15.45.
e. Responses to 10 ng. of 5HT (10) and to 200 μg. of acetylcholine (200) at 16.15.
f. Responses to 10 ng. of 5HT (10) and to a combined dose of 0.3 μ.M. of 5-benzyloxy-N-dimethyltryptamine plus 200 μg. of acetylcholine (0.3 + 200). The results suggest that 5-benzyloxy-N-dimethyltryptamine acts at the same receptors as 5HT but not at those acted upon by acetylcholine.

Time in min.

was 15 times more active than tryptamine, 5-methyl-α-methyltryptamine was 30 times more active than 5-methyltryptamine, 5-methoxy-α-methyltryptamine was 20 times more active than 5-methoxytryptamine, but 5-hydroxy-α-methyltryptamine was only half as active as 5-hydroxytryptamine. Also di-n-propyl-tryptamine was 20 times more active than tryptamine, but 5-hydroxy-N-di-n-propyltryptamine was only 1/16 as active as 5-hydroxytryptamine. The methoxy compounds were again, as on the rat uterus, more active than their 5-methyl analogues.

Of the 5-benzyloxy compounds, the 5-benzyloxy-α-methyl-tryptamine (14) was most active, but potency in this series, though declining in higher members, did not follow the same pattern as in the corresponding 5-hydroxy compounds. These compounds did not give a maximal response. To see if they acted on the same receptors as 5-hydroxytryptamine, a benzyloxy compound in a concentration which produced the biggest response obtainable, (of the N-diethyl compound, 6×10^{-5}M: of the N-dimethyl compound, 5×10^{-5}M) was added simultaneously with a dose of 5-hydroxytryptamine (100 ng. in a 5 ml. bath) which, by itself, produced a bigger effect than the 5-benzyloxy compound. In these circumstances (Fig.XVI) the response was smaller than that to the dose of 5-hydroxytryptamine alone.

When a dose of acetylcholine (200 µg, a large amount because there is hyoscine in the tyrode solution) was used in place of 5-hydroxytryptamine, there was no depression of the response to acetylcholine. This suggested that the 5-benzyloxy compounds occupied the same receptors as 5-hydroxytryptamine, but not those acted on by acetylcholine.

(iii) Depression of 5-hydroxytryptamine responses.

After stimulation with the compounds, 5-hydroxytryptamine and tryptamine, the preparation was insensitive to the small doses of 5-hydroxytryptamine for a variable time, depending on the dose and the type of drug used. It was more marked after the long-acting drugs like the α-methyl derivatives (other than 5-hydroxy-α-methyltryptamine) and the 5-benzyloxy compounds. When the dose response curves were being obtained, it was particularly necessary to allow time for this depression to pass off and to wait till the control responses to 5-hydroxytryptamine returned to normal before proceeding further.

3. EFFECTS ON THE GUINEA PIG ILEUM

Introduction. As already mentioned (p. 65) it was first necessary to establish that the tryptamine receptors in this preparation are affected similarly by both 5-hydroxytryptamine and tryptamine. To do this the dose ratios of tryptamine and morphine and

FIGURE E

Guinea pig ileum

Log. dose response curves to 5-hydroxytryptamine (HT) before and after the action of LSD 3×10^{-8}M.

Responses to HT (base) are shown as A (before the action of LSD) and B (after the action of LSD).

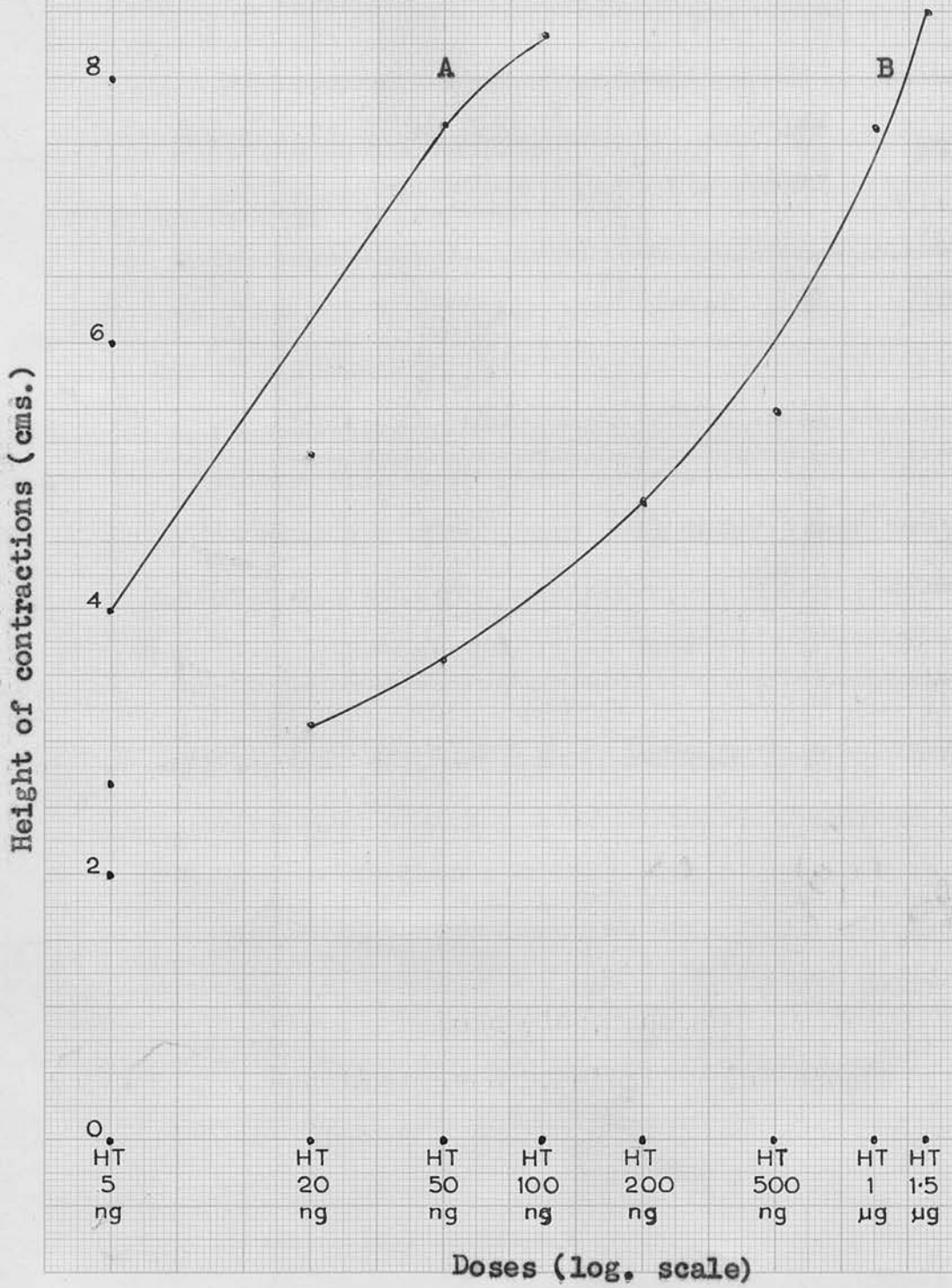


TABLE 7

Antagonistic activity of lysergic acid diethylamide, Dibenzyline and Morphine on the guinea pig ileum at 35°C

Compound No.	DRUG	No. of Expt	Conc. (M)	DOSE RATIO AT THE END OF 1HR. FOR:			
				5-Hydroxytryptamine	Tryptamine	Nicotine	Acetylcholine
1	Dibenzyline	5	3.4×10^{-7}	80-40 90-80 100-10 100-20 100	- - - - -	1 1.5 2 3 2	- - - - -
2	Lysergic-acid diethylamide	5	3×10^{-8}	40-20 50-35 10-8 10-8 25-15	80-40 40-25 40-25 13	1 1 1 1 -	1 1 1 1 1
3	Morphine	4	3.5×10^{-6}	3 ± 0.5(S.E.)	3 ± 0.5(S.E.)	3.5±0.4 S.E.	1 ± 0

- , means not tested (See text, page 95)

The two limits of the ratios for dibenzyline and lysergic acid diethylamide are given at the two extremes of the dose/response curve to 5-hydroxytryptamine and tryptamine (See text, page 95)

and tryptamine and lysergic acid diethylamide were compared with those of 5-hydroxytryptamine and the same antagonists. The results are shown in Table 7, and appear to be the same. Dibenzyline-treated tissue was so insensitive to the action of tryptamine, that no attempt was made to obtain dose ratios with tryptamine. The dose ratio figures during the presence of dibenzyline and lysergic acid diethylamide are given as a range because after the preparation was treated with these drugs, the dose/response curves to 5-hydroxytryptamine and tryptamine, although parallel with each other, were not parallel with the original curves (Fig. E). Gaddum and Picarelli (1957), (Gaddum, Personal Communication), had difficulty in obtaining dose ratios with lysergic acid diethylamide, because the base line was disturbed by this drug - it was for this reason that they used dibenzyline instead, to block the "D" receptors - but no great trouble of this sort was encountered in these experiments. The results seemed to indicate that tryptamine and 5-hydroxytryptamine acted at the same receptors in the guinea pig ileum. It was therefore, reasonable to proceed to test these compounds for their ability to imitate or antagonise 5-hydroxytryptamine on the 'tryptamine receptors' of this tissue.

TABLE 8

Antagonistic activity of some indoles on the "M" and "D" receptors in the guinea-pig ileum at 35°C

DOSE RATIO EXPERIMENTS

Compound No.	Drug	Conc. (M)	DOSE RATIOS AT THE END OF 1 HR. FOR:-						
			"D" receptors, in presence of Morphine					"M" receptors, after dibenzyline treatment	
			No. of expt.	5-HT	N	H	Ach.	No. of expt.	5-HT
41	5-Benzyloxy-gramine	3.6×10^{-7}	2	14 9	1 1	1 1	- -	1	1 -
		7.2×10^{-7}	1	15	3	1	-	-	-
32	5-Benzyloxy-N-dimethyl-tryptamine	6.2×10^{-6}	3	33 36 110	130 100 66	20 3 7	1 1 1	1	1 - -
3	N-dimethyl-tryptamine	6.6×10^{-6}	3	14 20 14	1 1 0.8	17 20 30	- - -	1	1 - -

5-HT = 5-hydroxytryptamine

N = Nicotine

H = Histamine

Ach. = Acetylcholine

- Means not tested.

On "M" receptors (Dibenzyline treated tissue) the analogues were maintained in the bath for only 15 min. as their effect was negative. The antagonistic action of N-dimethyltryptamine and 5-benzyloxy-N-dimethyltryptamine against 5-hydroxytryptamine were complete at the end of one hour, but their anti-histaminic effect was complete at the end of 15-20 min. only. 5-benzyloxygramine blocked the action of 5-hydroxytryptamine to a maximum extent after 20 min. contact, while the anti-nicotinic effect of 5-benzyloxy-N-dimethyltryptamine progressed beyond the end of one hour.

Only the compounds listed in Table 10 were tested (for both stimulant and antagonistic action) on this tissue. As in the rat uterus, their actions can be divided into:

(i) Antagonistic activity.

(ii) Stimulant activity.

(iii) Effects of higher concentrations.

(iv) Effect of temperature.

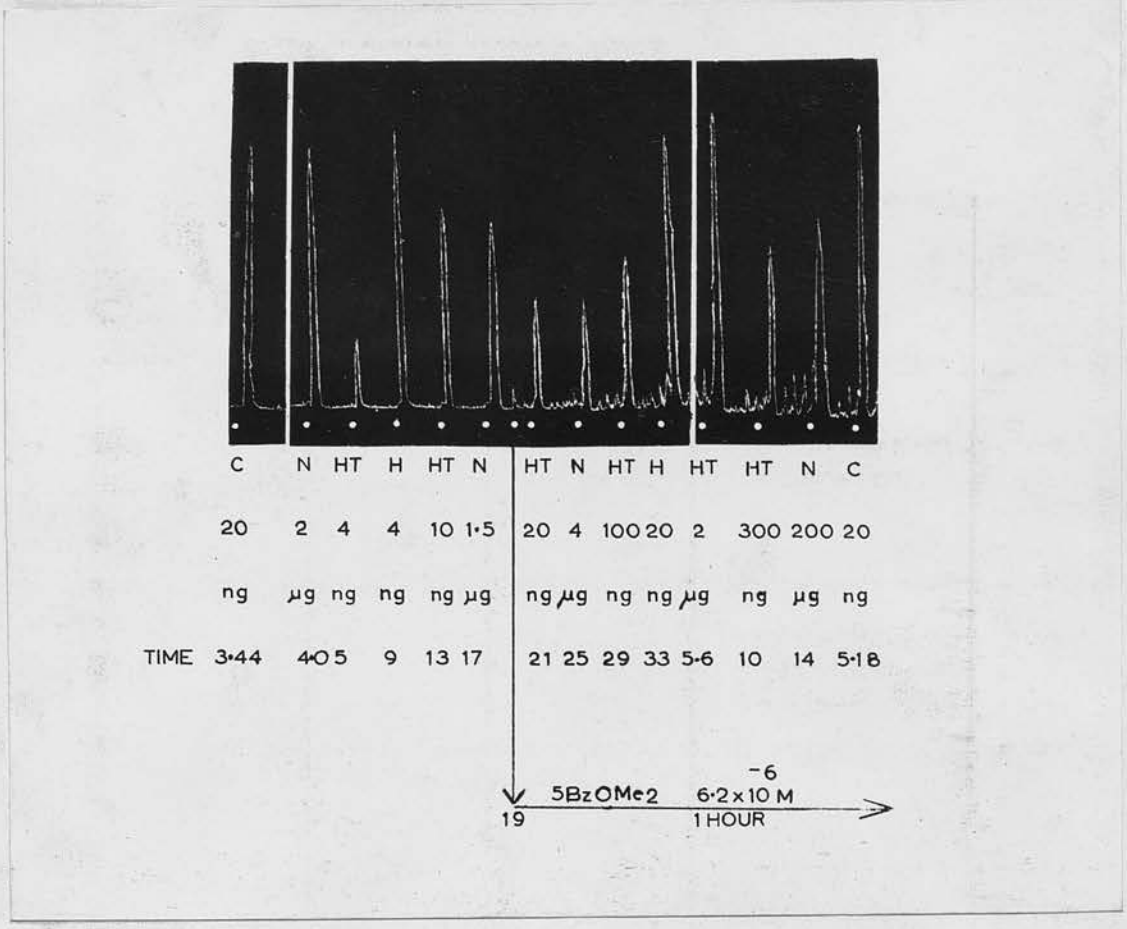
(v) Effects of increasing the concentration of morphine or lysergic acid diethylamide.

(i) Antagonistic activity. In the presence of morphine (i.e. on the "D" receptors which are thought to be in the muscle part of the gut), only three of the compounds examined had antagonistic activity. On the dibenzyline-treated tissue (i.e. the "M" receptors which are thought to be in the nervous part of the gut), none of the compounds, even of these three, had any antagonistic activity in the same concentration as in the test on the "D" receptors. These results are summarised in Tables 8 and 9.

Costa (1956) has reported that reserpine antagonised 5-hydroxytryptamine in the rat uterus and suggested that other substances, which are tranquillizers, might do the same. He showed that some compounds which produced hallucinations (e.g. lysergic acid diethylamide and mescaline) increased the

Figure XVII

Isolated guinea pig ileum preparation



Antagonistic effect of 5-benzyloxy-N-dimethyltryptamine ($5BzOMe_2$) on the "D" receptors (in the presence of morphine 3.5×10^{-6}M). Responses are shown to 5-hydroxytryptamine (HT, 4, 10, 20, 100, 300 ng. and 2 µg.); nicotine (N, 1.5, 2, 4 and 200 µg.); histamine (H, 4 and 20 ng.) and carbachol (C 20 ng.) before and after the action of analogue (6.2×10^{-6}M). At the end of 1 hr. the dose ratios are 33 for HT; 132 for N; 20 for H and 1 for C. The last response to H is not shown in the tracing.

TABLE 9

Antagonistic activity of some indoles on the "D" receptor in the presence of morphine on the guinea pig ileum at 35°C

DRUG RATIO EXPERIMENTS

Compound No.	DRUG	Conc. used (M)	No. of Expts	Mean drug ratio (molar basis) ± Standard Error)
41	5-Benzyloxy-gramine	3.6×10^{-7}	2	0.92 ± 0.28
		7.2	1	1.00
32	5-Benzyloxy-N-dimethyl-tryptamine	60	3	0.33 ± 0.1
3	N-dimethyl-tryptamine	66	3	0.055 ± 0.002

responses to 5-hydroxytryptamine in the rat uterus (p. 13). Gaddum (personal communication) considered that the "M" receptors in the guinea pig ileum (thought to be in the nervous part of the tissue), might be a more suitable model for testing the central activity of the compounds. Reserpine was therefore tested on the dibenzyline-treated tissue and was found to be without effect. This was disappointing, and indicated that this tissue could not be used to obtain an idea of the central action of the compounds.

Two interesting side-effects were observed in the guinea pig ileum treated with morphine ("D" receptors):

1) 5-benzyloxy-N-dimethyltryptamine (3) antagonised the effects of nicotine (Fig. XVII). These effects of nicotine have been termed "morphine-insensitive" by Kosterlitz and Robinson in 1959).

2) N-Dimethyltryptamine (3) and, to a lesser extent, 5-benzyloxy-N-dimethyltryptamine (32) antagonised the effects of histamine on this preparation.

(ii) Stimulant activity: The stimulant activity of the compounds tested is shown in Table 10. In the presence of morphine, contractions produced by all the stimulant compounds, including 5-hydroxytryptamine and tryptamine, were complete within 30-45 sec.

5-Hydroxytryptamine, tryptamine and 5-hydroxy-α-methyltryptamine (20) all appeared to be easily washed out, but after the other compounds a longer time (usually 2 min.) was required before the tissue relaxed. In concentrations used for plotting the dose/response curves the drugs produced a single contraction of the muscle (not repeated contractions). N-Dimethyltryptamine (3) caused slight depression (lasting about 5 min.) of the control responses to 5-hydroxytryptamine after a high concentration had been given, but after the other compounds the control responses were not disturbed.

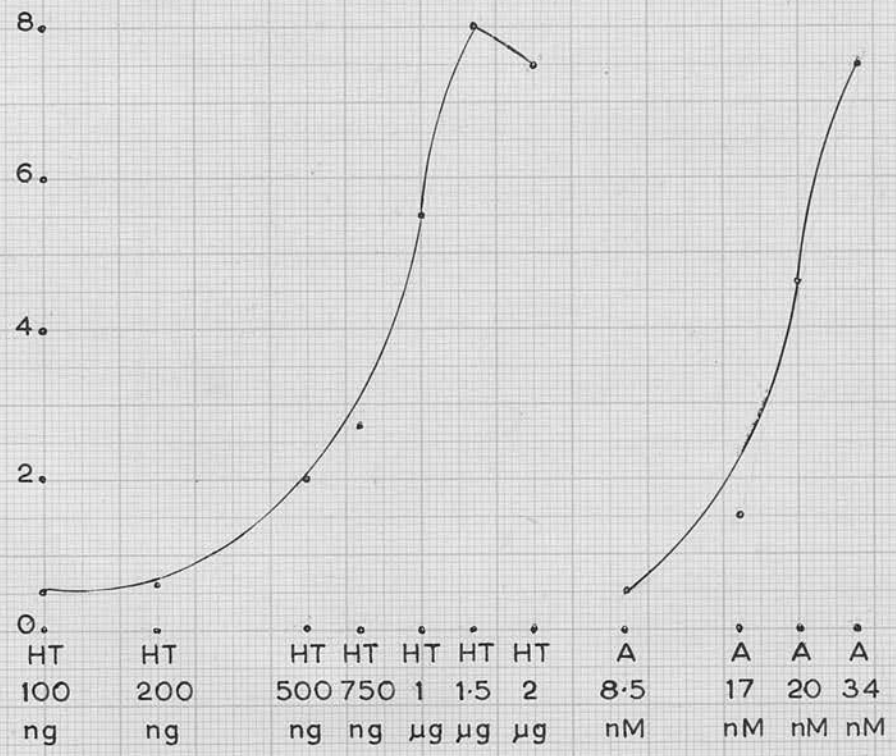
The dose/response curve of N-dimethyltryptamine (3), though parallel to those of 5-hydroxytryptamine and tryptamine in low concentrations, flattened out in higher concentrations (as Fig.B). The curves of the other active compounds were parallel to those of 5-hydroxytryptamine and tryptamine over the whole range. Of the compounds tested, 5-benzyloxygramine (41), 5-benzyloxy-N-diethyltryptamine (32) and α-ethyltryptamine (12) were inactive. α-Methyltryptamine (11) and N-dipropyltryptamine (5) were about as active as tryptamine, and 5-hydroxy-α-methyltryptamine (20) was about half as active as 5-hydroxytryptamine.

On the preparation treated with dibenzyline, 5-

FIGURE F

Guinea pig ileum (LSD 3×10^{-8}M in Tyrode

Log. dose response curves to 5-hydroxytryptamine (HT) and 5-hydroxy-α-methyltryptamine (A) on "M" receptors.



Height of contractions (cms.)

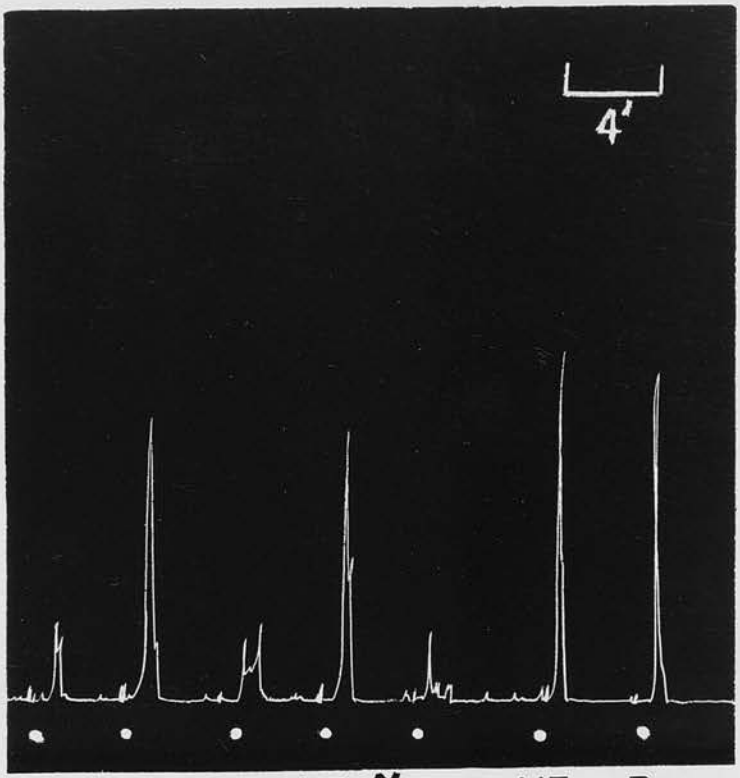
Dose (log. scale)

HT (ng. and µg. base; 100ng.=55 p.Moles)

A (n Moles)

FIGURE XVIII

Isolated guinea pig ileum preparation



Action on "M" receptors. (Preparation treated with dibenzyline). Responses are shown to 5-hydroxytryptamine (HT 250 and 500 ng.), tryptamine (T, 200 µg.), α-methyltryptamine (α-Me T, 800 n. Moles) and N-dipropyltryptamine (nPr_2 710 n. Moles). Time in min.

HT 500 ng. = 275.0 p. Mole

T 200 µg. =

hydroxy-α-methyltryptamine (20) was the only compound which was active enough for a dose/response curve to be determined. This was parallel to that of 5-hydroxytryptamine (Fig. F). The other compounds tested only produced small effects even in very high concentrations (Fig. XVIII).

In the presence of lysergic acid diethylamide dose/response curves were obtained with a few compounds (Table 10). 5-Hydroxy-α-methyltryptamine (20) was again the most active and its dose/response curve was parallel to those of 5-hydroxytryptamine and tryptamine. N-Dipropyl- and 5-hydroxy-N-dipropyl- tryptamines (5, 23) were the next most active, being stronger than tryptamine, but their dose/response curves flattened out at high concentrations. The effects of these latter compounds can only be shown on the "M" receptors when the "D" receptors are blocked by lysergic acid diethylamide. Dibenzyline leaves the tissue so insensitive (even to 5-hydroxytryptamine) that responses to the less active compounds could only be expected with very high concentrations indeed.

One of the most striking features of Table 10 is the high activity of N-dipropyltryptamine (5) on the "M" receptors. This resembles the results on the rat fundus strip although on the latter preparation

TABLE 10

Stimulant activity of some indoles on the guinea pig ileum at 35°C

EQUIPOTENT MOLAR RATIOS.

Compound No.	DRUG	"D" RECEPTORS		"M" RECEPTORS			
		In the presence of Morphine 3.5×10^{-6}M		Dibenzyline treated		In the presence of ISD 3×10^{-8}M	
		Mean ratio ± S.E.	No. of Expt	Mean ratio ± S.E.	No. of Expt	Mean ratio ± S.E.	No. of Expt
1	5-Hydroxy-tryptamine	1		1		1	
2	Tryptamine†	162 ± 40	8	360,720+	2	257 ± 30	5
23	5-Hydroxy-N-dipropyl-tryptamine	12, 20	2	120 ± 0+	2	†12.5, 25	2
20	5-Hydroxy-α-methyl-tryptamine	2.3 ± 0.03	3	4, 16	2	3.2, 8	2
5	N-dipropyl-tryptamine	150 ± 53	4	148, 296+	2	†10 ± 3.6	4
11	α-Methyl-tryptamine	370 ± 110	3	>200,640+	2	390 ± 125+	3
12	α-Ethyl-tryptamine	>20,000	1	>200	1	-	
3	N-diethyl-tryptamine	†130 ± 40	3	100+	1	-	
32	5-Benzyloxy-N-dimethyl tryptamine	5000, >2000+	2	>200	1	-	
41	5-Benzyloxy-gramine	>5000	1	>200	1	-	

† indicates the dose/response curve was not parallel to that of 5-hydroxy-tryptamine.
+means just threshold responses, no idea of the dose/response curve.
-means not tested.
†Equipotent molar ratio was 140 ± 7 (S.E.) (5)Expt. during normal Tyrode

the dose/response curve is parallel to that of 5-hydroxytryptamine over the whole range.

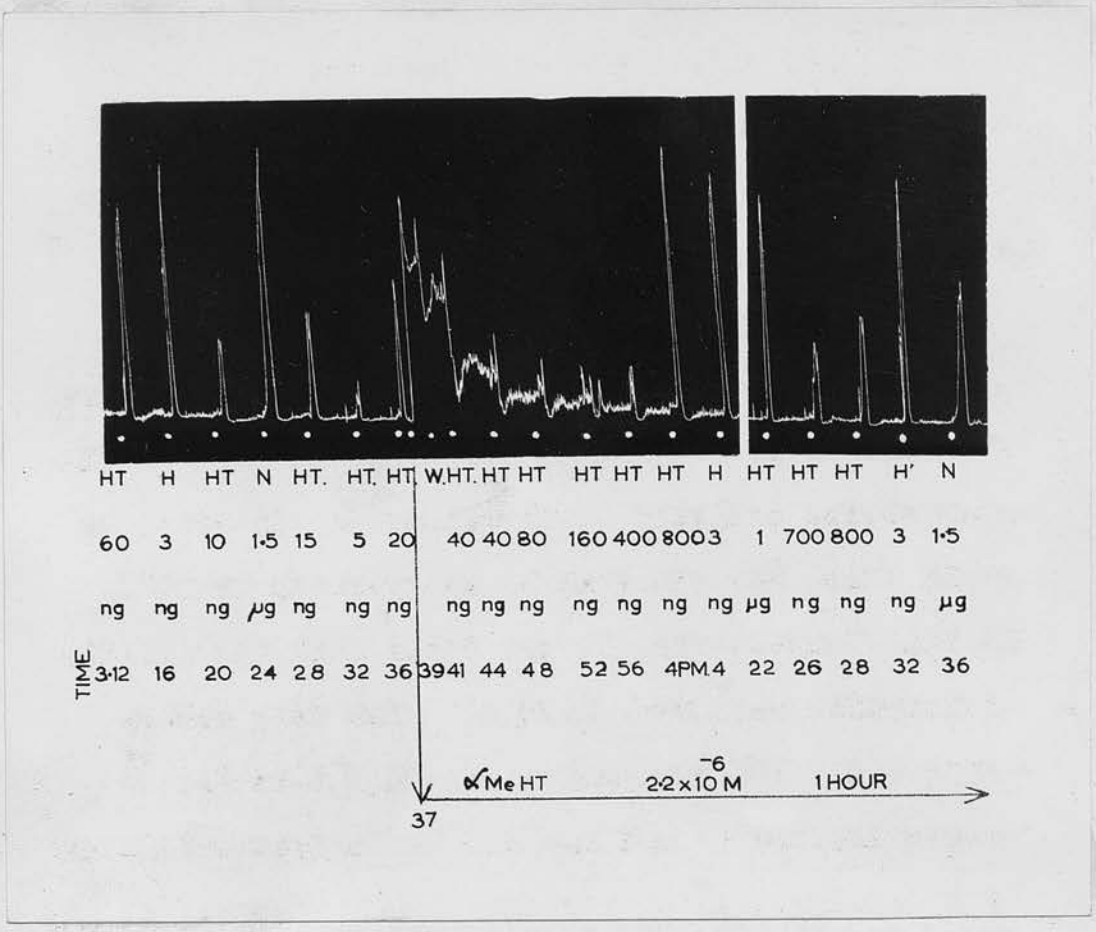
One of the most puzzling results in Table 10 is the activity of tryptamine relative to 5-hydroxytryptamine. This does not appear to be the same on the "M" and "D" receptors. Further, the activity of 5-hydroxytryptamine on the normal gut is less than would be expected, if 5-hydroxytryptamine and tryptamine act only on the "M" and "D" receptors. These results may merely emphasise the errors involved in these experiments, or they may indicate that 5-hydroxytryptamine and tryptamine act differently at the different receptors, and even suggest the existence of other receptors in the tissue, where these drugs may act. Whatever the answer may be, the situation is obviously extremely complicated and the idea of testing all the compounds on this tissue was dropped.

(iii) Effects of higher concentrations. On this preparation, either in presence of morphine or on the dibenzyline-treated tissue, there was no indication of the production of repeated contraction such as was seen on the rat uterus. A multiple response was sometimes seen but the tissue rapidly relaxed in spite of the presence of the drug.

The preparation was then insensitive to 5-

Figure XIX

Isolated guinea pig ileum preparation



Test for repeated contractions and tachyphylaxis on the "D" receptors (in the presence of morphine 3.5 x 10^{-6}M). The analogue used is 5-hydroxy-α-methyltryptamine, αMeHT, 2.2 x 10^{-6}M). Responses are shown to 5-hydroxytryptamine (HT, 5, 10, 15, 20, 40, 60, 80, 160, 400, 700, 800 ngs. and 1 µg.). Histamine (H, 3 ng.); nicotine (N, 1.5 µg.) before and after treatment with the analogue. No repeated contractions, like those seen on the rat uterus, were produced and the base line assumed normal level. At the end of 1 hr. there was a marked depression of the responses to HT but no significant effect against H and N.

hydroxytryptamine and tryptamine (even if the drug was washed out) but the control responses to nicotine, acetylcholine and histamine were unaffected. This type of effect was also produced when lower concentrations (below those producing a maximal response) were left in for a long time (more than 5-10 min.) (Fig.

(iv) Effect of temperature. In the first experiments on the guinea pig's ileum, the temperature was 35°C. and the work was complicated by the spontaneous activity of the tissue. In order to avoid this, the temperature was reduced to 28°C. At this temperature, it was found that the effects of morphine were very feeble. The dose ratios using 3.5×10^{-6} M morphine were 1.6 ± 0.36 for 5-hydroxytryptamine and 1.4 ± 0.4 for tryptamine (mean of 6 experiments $\pm$ the standard error) whereas at 35°C. they were 3.0 ± 0.5 and 3.0 ± 0.5 (Table 7). This result was surprising. Kosterlitz and Robinson (1957 and 1958) and Innes, Kosterlitz and Robinson (1957) found that contractions of the longitudinal muscle of the guinea pig ileum in the "preparatory phase" of the peristaltic reflex, and contractions produced by nicotine, barium ions, or 5-hydroxytryptamine were all reduced by morphine, atropine, or lowering the bath temperature. It might therefore be expected that responses to 5-hydroxytrypta-

mine would be reduced more by morphine and lowering the temperature together than by either dose. The results suggest that the actions of morphine and of lowering the temperature may be similar.

v) Effect of increasing the concentration of morphine and lysergic acid diethylamide. Kosterlitz and Robinson (1955,1958) showed that increasing the concentration of morphine above a certain level (1.7×10^{-7} M) did not increase the antagonism: a concentration 1,000 times as big (8×10^{-5} M) did not produce any greater antagonism of 5-hydroxytryptamine, nicotine or barium ions; Gaddum and Picarelli (1957) found that with 3.5×10^{-6} M morphine "the "M" receptors appear to be more or less completely blocked". Gaddum and Hameed (1954) have shown that increasing the concentration of lysergic acid diethylamide from 3×10^{-8} M to 3×10^{-5} M did not increase the antagonism of 5-hydroxytryptamine.

These experiments were repeated using tryptamine, nicotine, and acetylcholine as well as 5-hydroxytryptamine and it was found that tryptamine behaved like 5-hydroxytryptamine. The concentration of morphine used (3.5×10^{-6} M) produced a maximal antagonism of 5-hydroxytryptamine, tryptamine and nicotine but had no effect on responses to acetylcholine. A ten-fold increase in the concentration of morphine did not increase the

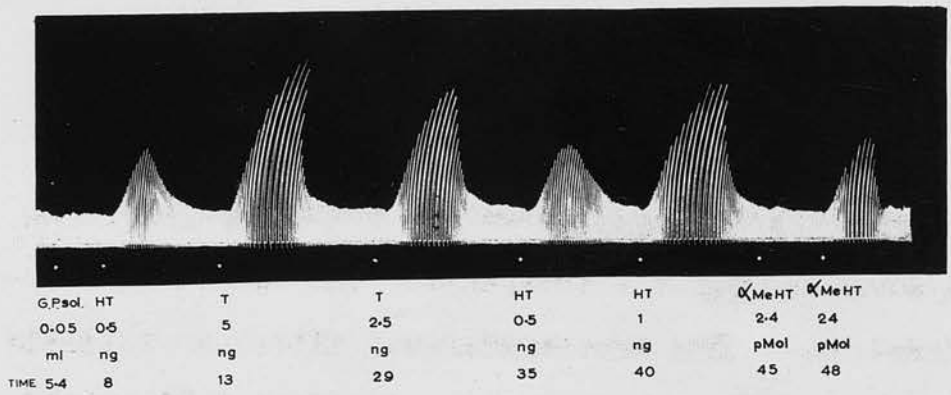
TABLE 11

Stimulant activity of some compounds on the perfused rabbit ear preparation

Compound No.	DRUG	Equipotent molar ratios ± S.E.	No. of Expts
1	5-Hydroxytryptamine	1	
2	Tryptamine	6 ± 1.7	3
20	5-Hydroxy-α-methyl tryptamine	6 ± 1.2	3
5	N-dipropyltryptamine	10,000	2
16	2-Methyl-N-dimethyl-tryptamine	>50,000	2
	Adrenaline	3 ± 1	3

Figure XX

Isolated perfused rabbit's ear preparation



Effect of 5-hydroxy-α-methyltryptamine (αMeHT). Responses are shown to 5-hydroxytryptamine (HT, 0.5 and 1 ng.); tryptamine (T, 2.5 and 5 ng.), and αMeHT 2.4 and 24 P Moles. 0.05 ml. of Green and Page solution (G.P.sol.) had no effect.

HT 1 ng. = 0.55 p Mole.

T 5 ng. = 3.7 p Moles.

antagonism of 5-hydroxytryptamine, tryptamine and nicotine (and had no effect against acetylcholine).

The concentration of lysergic acid diethylamide used ($3x10^{-8}$ M) produced maximum antagonism of 5-hydroxytryptamine and tryptamine but had no effect against nicotine.

4. Effects on the perfused rabbit's ear.

Only the compounds shown in Table 11 were tested on this preparation. Small doses of 5-hydroxytryptamine, tryptamine, 5-hydroxy-α-methyltryptamine (20) and adrenaline, all caused vasoconstriction. The vasoconstrictor effect of moderate doses of these compounds passed off in 3-10 minutes.

The dose response curves seemed to be parallel (Fig.XX) although there is only a small range of doses which can be given, which makes it difficult to judge exactly. Equipotent molar ratios were determined and are shown in Table 11. N-Dipropyltryptamine (5) and 2-methyl-N-dimethyltryptamine (16) were practically without vasoconstrictor properties, but both compounds antagonised the effects of 5-hydroxytryptamine, tryptamine and adrenaline. The N-dipropyltryptamine (5) showed this effect in the range of 35 n Mol to 525 n Mol, and was more active an antagonist than the 2-methyl-N-dimethyltryptamine (16). About 50 minutes after a single

262 n Mol dose of N-dipropyltryptamine (i.e. just before recovery commenced), the dose ratios for 5-hydroxytryptamine and adrenaline were 100, 200 and 10 respectively when the recovery process started. After 10 min. after a 420 n Mol dose of 2-methyl-N-dimethyltryptamine (16), the dose ratios for 5-hydroxytryptamine and adrenaline were about 10 each.

5. DIFFERENCES BETWEEN THE EFFECTS OF 5-HYDROXYTRYPTAMINE AND TRYPTAMINE

(i) Introduction. In the course of testing the antagonistic properties of the compounds it seemed that some substances antagonised 5-hydroxytryptamine more than they did tryptamine. This effect was, therefore, studied by determining the dose ratios for 5-hydroxytryptamine, tryptamine and acetylcholine of the compounds concerned. On the rat uterus (Table 2) one compound, 2-methyl-N-dimethyltryptamine (16) in a concentration of 10^{-5} M potentiated the effects of tryptamine and acetylcholine but not those of 5-hydroxytryptamine (Fig.VIII). With the other compounds, however, there is not a convincing difference between the dose ratio figures for 5-hydroxytryptamine and tryptamine (Table 2).

On the rat fundus strip, the situation is clearer. 2-Methyl-N-dimethyltryptamine (16), N-dimethyltryptamine (3), 5-benzyloxy-N-dimethyltrypta-

mine (32), 5-benzyloxygramine (41), and also bromolysergic acid diethylamide are much more effective antagonists of 5-hydroxytryptamine than of tryptamine (Table 6). These findings might be taken to mean that, even though the dose response curves for 5-hydroxytryptamine and tryptamine are parallel on both the rat uterus and rat fundus strip, 5-hydroxytryptamine and tryptamine act at different receptors, and that the compounds listed in Table 6 block the 5-hydroxytryptamine receptors much more than they block the tryptamine receptors. This idea was suggested by Woolley and Shaw (1957c) to explain their finding that BAS (42) and BAS phenol (see page 42) antagonised the effects of 5-hydroxytryptamine much more than those of tryptamine on the blood-pressure of anaesthetised dogs.

Another possible explanation is that 5-hydroxytryptamine and tryptamine act at the same receptors but that the compounds both block the receptors and simultaneously potentiate tryptamine. This would necessitate an action by the compounds on the receptors in the same concentration as on an enzyme destroying tryptamine (but not 5-hydroxytryptamine).

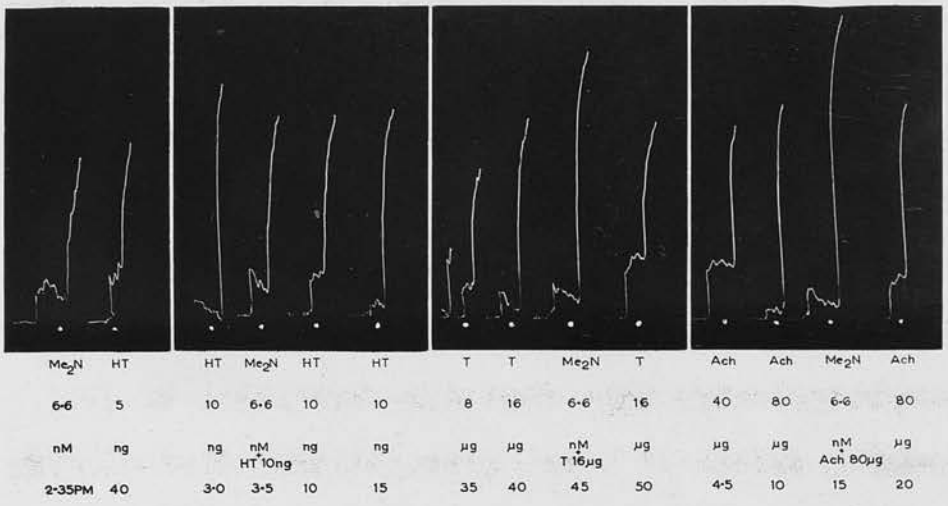
(ii) Differences between the stimulant actions of the other compounds. It seemed that on the rat fundus strip, the actions of 5-hydroxytryptamine and tryptamine were different, so an attempt was next made to analyse the stimulant activity of some of

the other compounds by observing the effects on this activity of a substance which antagonised 5-hydroxytryptamine rather than tryptamine. 2-Methyl-N-dimethyltryptamine (16) and N-dimethyltryptamine (3) were not really ideal for this purpose because they could themselves cause contractions but bromo-lysergic acid diethylamide seemed quite suitable (Table 5). BAS (42), which might be regarded as an obvious choice, was almost inactive on the rat fundus strip.

Bromo-lysergic acid diethylamide was usually used in a concentration of 1.2 to 3.7 x 10^{-7} M. A concentration of 1.2 x 10^{-7} M produced a dose ratio greater than 10 for 5-hydroxytryptamine and less than 10 for tryptamine. Its effect took about 30 min. to develop and the preparation was used when it had been exposed to the action of bromo-lysergic acid for 1 hr. If the superfusion fluid was changed back to normal tyrode, the preparation recovered in about 1 hr. Higher concentrations of bromo-lysergic acid diethylamide increased the dose ratio of 5-hydroxytryptamine without increasing that of tryptamine. The significance of this is discussed later (p.119). These concentrations of bromo-lysergic acid diethylamide, unlike similar concentrations of lysergic acid diethylamide, did not disturb the base line, but the contraction of the

Figure XXI

Rat fundus strip preparation



Effects of N-dimethyltryptamine (Me_2N) on responses to 5-hydroxytryptamine (HT); tryptamine (T) and acetylcholine (Ach.). Responses are shown to Me_2N (6.6 n. Moles); HT (5 & 10 ng.); T (8 and 16 µg.) and ach. (40 and 80 µg.) alone and the combined doses of Me_2N (6.6 n. Moles) with HT, 10 ng; T, 16 µg. and Ach. 80 µg. Note that N-dimethyltryptamine reduces the effect of 5-hydroxytryptamine but increases those of T and Ach.

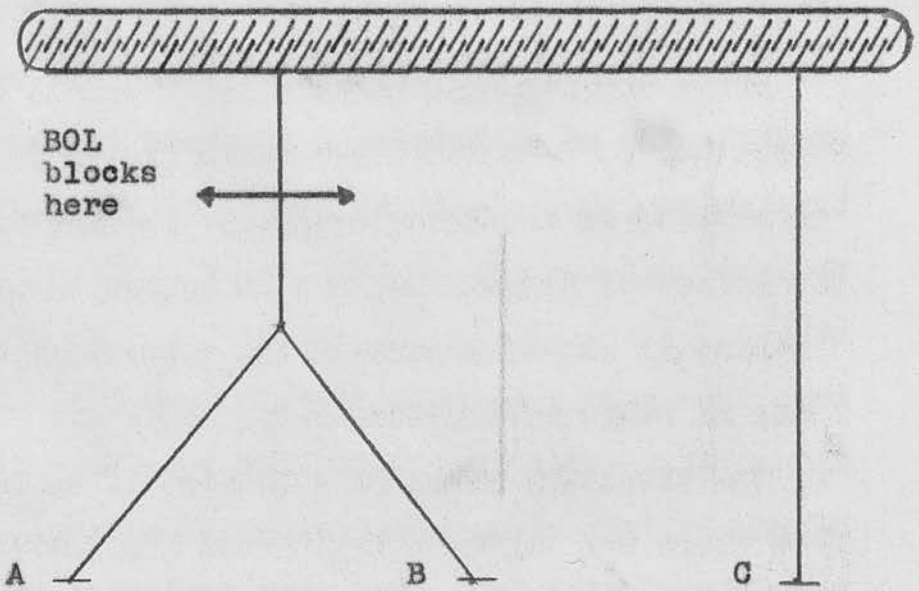
Hyoscine 2.3 and 10^{-7}M in Tyrode solution.

muscle, even in response to 5-hydroxytryptamine and tryptamine took longer to develop. In these experiments, a period of 3 min. was allowed (instead of 90 sec.) for the contraction to be completed.

Table 5 shows the effects of bromo-lysergic acid diethylamide on contractions produced by 5-methyltryptamine (30), N-dimethyltryptamine (3) and N-dipropyltryptamine (5) as well as by 5-hydroxytryptamine and tryptamine. It seemed that the contractions produced by N-dimethyltryptamine (3), and N-dipropyltryptamine (5) were like those produced by 5-hydroxytryptamine, whereas the effects of 5-methyltryptamine (30) were more resistant to the blocking effect of bromo-lysergic acid diethylamide and thus resembled the effects of tryptamine.

Further evidence that the contractions produced by N-dimethyltryptamine (3), were caused by an action at the receptors where 5-hydroxytryptamine acted, was obtained by the following experiments. Responses were obtained with N-dimethyltryptamine (3), 5-hydroxytryptamine 10 ng., tryptamine 10 µg. and acetylcholine 100 µg (a high dose because of the presence of hyoscine in the tyrode solution (see p. 53). When 6.6 n Mol of N-dimethyltryptamine (3) was added together with 10 ng of 5-hydroxytryptamine, the response was less than that previously obtained by 10 ng. of 5-hydroxytryptamine

Three types of receptors in the rat fundus strip



Drugs	5-Hydroxytryptamine (HT)	N-Dimethyl tryptamine (NMe_2)	Tryptamine (T)
BOL	Blocks	Blocks	No block
HT	Stimulates	?	No effect
T	Stimulates	?	Stimulates
NMe_2	Low concentration blocks	High concentration stimulates	No effect

(Fig. XVI), but when 6.6 n Mol of N-dimethyltryptamine (3) was added together with either 10 µg of tryptamine or 100 µg of acetylcholine, the responses were significantly increased.

These results were surprising. On the rat fundus strip, N-dimethyltryptamine (3) antagonised the effects of 5-hydroxytryptamine (without altering the effect of tryptamine) but in higher concentrations it caused contractions, apparently like those of 5-hydroxytryptamine.

The situation could be explained by supposing that there are separate tryptamine and 5-hydroxytryptamine receptors and either that substances like N-dimethyltryptamine (3) stimulate in high concentration, a receptor, which they block in low concentrations; or that the 5-hydroxytryptamine receptors are of two types; one of which is blocked by N-dimethyltryptamine (3) in low concentrations and the other stimulated by N-dimethyltryptamine (3) in high concentrations. This scheme is illustrated in Fig. opposite. Further, from the experiments with bromo-lysergic acid diethylamide described on page

it would even seem possible that there are two types of tryptamine receptors (because increasing the concentration of bromo-lysergic acid diethylamide above 3.7×10^{-7} M did not increase the block of tryptamine). It is difficult to see why a

substance should stimulate in high concentration a receptor which it blocks in low concentration, but before accepting the existence of two types of 5-hydroxytryptamine receptors, it was decided to see what would happen if a concentration of N-dimethyltryptamine (3) which caused stimulation was applied to a preparation which was already under the influence of the blocking action of a lower concentration of the same substance. The result obtained is shown in Fig.XII and there is no doubt that a low concentration applied for a long time, antagonises the effects of a high concentration applied for a short time. In these circumstances, there is no reason to suppose the existence of two types of 5-hydroxytryptamine receptors.

(iii) Are there separate tryptamine and 5-hydroxytryptamine receptors? At this stage of the work, the results could be explained either by supposing that there were separate tryptamine* and 5-hydroxytryptamine receptors in the rat fundus strip or that there was only one type of receptor

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*The possibility of there being two types of T receptors, was mentioned on p. and is discussed in more detail on p.

and that the antagonists studied blocked this receptor at the same time as they blocked the destruction of tryptamine.

Vane (1959) showed that the amine-oxidase inhibitors, marsilid and phenylisopropyl hydrazine, potentiated the actions of some compounds, including tryptamine, on the rat fundus strip but not the actions of other compounds, including 5-hydroxytryptamine. The compounds potentiated were all substances which contained an oxidizable amino group but did not contain a free phenolic hydroxyl group. Vane showed that finely ground suspensions of rat fundus would oxidise both 5-hydroxytryptamine and tryptamine and suggested that tryptamine and compounds like it, which lacked a free phenolic group but contained an oxidizable NH_2 group, were destroyed inside the cells by amine oxidase and so potentiated by marsilid. 5-hydroxytryptamine and other phenolic substances with an oxidizable NH_2 group were not potentiated by marsilid and were not destroyed because they did not penetrate the cell. Substances which had no oxidizable NH_2 group were likewise not potentiated by marsilid and not destroyed by amine oxidase.

The compounds in Table 5, N-dimethyltryptamine (3) and N-dipropyltryptamine (5) which produced contractions blocked by bromo-lysergic acid diethyl-

TABLE 12

Stimulant activity of some indoles on the rat fundus strip during the presence of Marsilid 3 x 10^{-5}M in tyrode solution at 37°C

Compound No.	DRUG	No. of Expt	Mean equipotent molar ratio ± S.E.	x times differences in equipotent molar ratio during the presence of Marsilid	
				In this work	Vane (1959)
1	5-hydroxy-tryptamine	-	-	-	No difference
2	Tryptamine	5	26 ± 2.7	36 times as active	12.7 times as active
30	5-Methyl-tryptamine	3	14 ± 1.5	44 times as active	20 times as active
3	N-Dimethyl-tryptamine	3	902 ± 142	Half as active	1.8 times as active

Effect of Marsilid on 5-hydroxytryptamine and tryptamine responses on the rat uterus at 30°C

DOSE RATIO EXPERIMENTS

Conc.(M) of Marsilid used	No. of Expts	DOSE RATIOS AT THE END OF 45 MIN. FOR:		
		5-Hydroxytryptamine	Tryptamine	Acetylcholine
4 x 10^{-7}	1	1	1	1
12	1	1	1	0.75
22	1	1	1	1
30	1	0.5	0.5	1
75	2	1	0.5	1
		1	0.75	0.75
300	1	1	0.5	1

amide, in a way similar to those produced by 5-hydroxytryptamine, were not potentiated by marsilid (Vane, 1959). Contractions produced by 5-methyltryptamine (30) however, resembled those produced by tryptamine, they were less easily blocked by bromo-lysergic acid diethylamide than those of 5-hydroxytryptamine and were potentiated by marsilid.

Table 12 shows results obtained which confirmed Vane's (1959) findings. In these experiments, marsilid x 10^{-5} M was added to the tyrode (which already contained hyoscine 2.3 x 10^{-7} M). The contractions caused by 5-hydroxytryptamine (doses of 10-20 ng. were used) were unaffected. They were complete within 90 sec. and the base line was normal after about 1 min. stretching. Contractions caused by N-dimethyltryptamine (3) were also unaffected by marsilid but, as on the normal preparation, took 120 sec. for completion and required 2-3 min. stretching for recovery. Contractions caused by tryptamine and 5-methyltryptamine (30) were potentiated by marsilid and required longer time for stretching and recovery than in normal tyrode. They resembled the contractions produced

TABLE 13

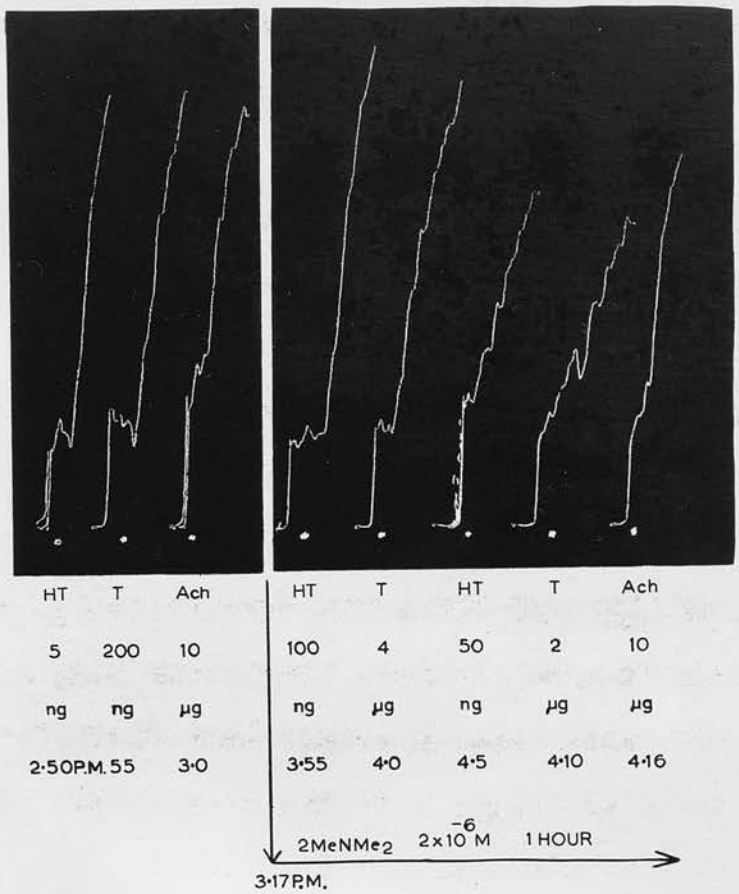
Antagonistic activity of bromo-lysergic acid diethylamide and some analogues to 5-hydroxytryptamine, 5-methyltryptamine and tryptamine responses on the rat fundus strip during the presence of Marsilid (3×10^{-6}M) in tyrode solution, at 37°C. (Dose ratio experiment).

Compound No.	DRUG	Conc. (M)	DOSE RATIOS AT THE END OF 1HR. FOR:-			
			5-Hydroxy-tryptamine	Trypt-amine	5-Methyl tryptamine	Acetyl-choline
	Bromo-lysergic acid diethylamide	1.2×10^{-7}	10	10	10	1
			12.5	20	-	1
		3.7	800	1000	-	0.75
			200	200	200	1
16	2-Methyl-N-di-methyltryptamine	20	15	15	-	1
			4	5	-	1
3	N-dimethyl-tryptamine	3.3	13	20	-	1
			20	20	-	1

- means not tested.

Figure XXII

Rat fundus strip preparation



Antagonistic effect of 2-methyl-N-dimethyltryptamine ($2MeNMe_2$) in the presence of Marsilid 3×10^{-5}M in Tyrode solution. Responses are shown to 5-hydroxytryptamine (HT, 5, 50 and 100 ng.); tryptamine (T, 200 ng. 2 and 4 µg.) and acetylcholine (Ach. 10 µg.) before and after the action of the analogue in a concentration of 2×10^{-6}M for 1 hr. Note that at the end of the experiment, the responses to both 5-HT and T are equally depressed.

Hyoscine 2.3×10^{-7}M used in Tyrode solution.

by N-dimethyltryptamine (3)*.

It seemed possible therefore, that the results could all be explained by supposing that substances which blocked 5-hydroxytryptamine more than tryptamine were really only blocking one receptor (affected by either 5-hydroxytryptamine and tryptamine) and simultaneously blocking amine oxidase and thus potentiating tryptamine.

If this were so, the substances which antagonised 5-hydroxytryptamine more than tryptamine should antagonise both equally when an amine oxidase inhibitor was present. Table 13 shows dose ratios obtained in the presence of marsilid. Bromo-lysergic acid diethylamide, 2-methyl-N-dimethyltryptamine (16) and N-dimethyltryptamine (3) antagonised 5-hydroxytryptamine, tryptamine and, in two experiments with bromo-lysergic acid diethylamide, 5-methyltryptamine (30), to the same extent, thus supporting the idea set out above.

The experiment described on page 111, in which N-dimethyltryptamine (3) was added in a high dose to 5-hydroxytryptamine, tryptamine and acetylcholine was repeated in the presence of marsilid. Con-

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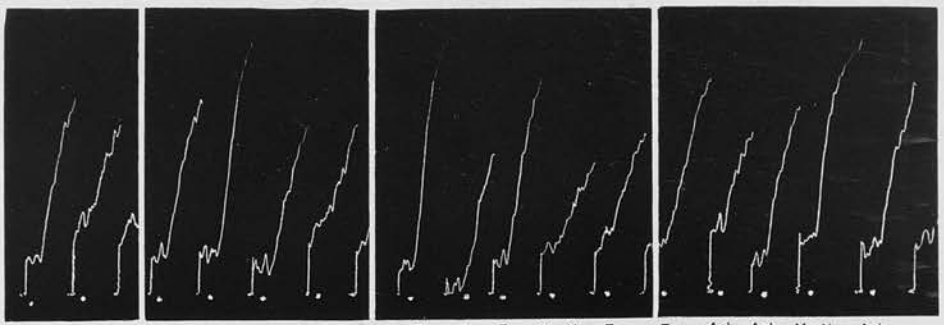
*Control responses to 5-hydroxytryptamine were not disturbed in these experiments by tryptamine and 5-methyltryptamine (30) but after N-dimethyltryptamine (3), they were depressed for 15-30 min. This is much longer than the depression (usually lasting 5 min.) caused by similar doses in the absence of marsilid. This may be caused (at least in part) by the need to use twice the dose when marsilid is present.

Figure XXIII

Rat fundus strip preparation



Effects of N-dimethyltryptamine (Me_2N) on response to 5-hydroxytryptamine (HT); tryptamine (T) and acetylcholine (Ach.) in the presence of Marsilid 3×10^{-5}M in Tyrode solution.

Responses are shown in Me_2N (33 nM), HT (10 and 20 ng.); T (200 and 500 ng.) and Ach. (5 and 10 µg.) alone and the combined doses of Me_2N (33 nM) with HT 20 ng; T, 500 ng. and Ach. 10 µg. Note the N-dimethyltryptamine reduces the effects of HT and T to an equal extent but increases that of Ach.

Hyoscine 2.3×10^{-7}M used in Tyrode solution.

tractions were produced with 20 ng. of 5-hydroxy-tryptamine, 500 ng. of tryptamine and 10 µg. of acetylcholine. N-Dimethyltryptamine (3) (33 n Mol) produced a contraction and when added at the same time as the doses of 5-hydroxytryptamine or tryptamine reduced the response, whereas it increased the response to acetylcholine (Fig.XXIII). This result again supports the idea that there is only one receptor and that some compounds antagonise 5-hydroxytryptamine more than tryptamine because they decrease the destruction of tryptamine at the same time as blocking the receptors.

iv. Are there two tryptamine-receptors in the rat fundus strip? Although in the rat fundus strip the evidence so far is that the 5-hydroxytryptamine is a tryptamine receptor, there remains the possibility from the experiments described on page 112, that there may be two types of tryptamine-receptors, one, blocked by bromo-lysergic acid diethylamide in concentration from 1.2 - 3.7 x 10^{-7} M and apparently the receptor where 5-hydroxytryptamine acts, and another which is not blocked by bromo-lysergic acid diethylamide.

In the guinea pig ileum there are also supposed to be two types of receptors. On one, the responses to 5-hydroxytryptamine are blocked by bromo-lysergic

acid diethylamide (Gaddum and Picarelli, 1957) and it has been shown (p. 95) that, on this receptor, the responses to tryptamine are similar to those of 5-hydroxytryptamine.

By analogy, the second type of receptor in the rat fundus strip might be compared with the morphine sensitive receptor in the guinea pig ileum. However, morphine did not antagonise the effects of 5-hydroxytryptamine and tryptamine on a preparation treated with a high concentration of bromo-lysergic acid diethylamide (2.5×10^{-6} M). In this experiment, hyoscine was omitted from the tyrode. As a check, the effects of bromo-lysergic acid diethylamide on the responses to 5-hydroxytryptamine and tryptamine were studied using normal tyrode and found to be the same as before. From these results, it did not appear that the second type of tryptamine-receptor in the rat fundus strip could be compared with the morphine sensitive ("M" type) receptor of the guinea pig ileum.

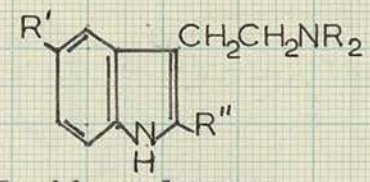
SECTION IV

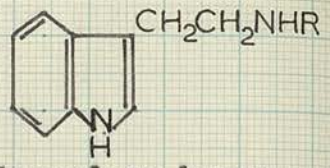
DISCUSSION

FIGURE H

Pictorial summary of results in Table 1

Stimulant activities of indoles on the rat fundus strip.





Secondary bases = ●

Tertiary bases:-

- ● R′ = R″ = H
- ● R′ = H, R″ = Me
- ● R′ = BzO, R″ = H
- ● R′ = HO, R″ = H

Equipotent molar ratios. (log. scale)

61536
4096
256
16
0

R= H Me Et isoPr Pr Bu

TABLE 14

Antagonists of 5-hydroxytryptamine in the order of their activity

ON RAT UTERUS	ON RAT FUNDUS STRIP
5-Benzyloxygramine+	N-Dimethyltryptamine
5-Benzyloxy-α-methyltryptamine	5-Benzyloxy-N-dimethyltryptamine
5-Benzyloxy-N-dipropyltryptamine	2-Methyl-N-dimethyltryptamine
5-Benzyloxy-N-diisopropyltryptamine	5-Benzyloxygramine
5-Benzyloxy-N-diethyltryptamine	
5-Hydroxy-N-dibutyltryptamine	
5-Benzyloxy-N-dimethyltryptamine+	
5-Benzyloxy-N-morpholinotryptamine	
N-Dimethyltryptamine+	
2-Methyl-N-dimethyltryptamine	

+These compounds were also tested and found to be antagonists of 5-hydroxytryptamine on the "D" receptors (in the presence of morphine), of the guinea pig ileum. The order of their activity was the same as on the rat uterus, but had no such effect on the "M" receptors (dibenzyline treated) of the guinea pig ileum.

DISCUSSION

The compounds with any degree of antagonistic activity are summarised in Table 14 and the variation of stimulant activity with structure is summarised in Figs. G and H.

In the 5-hydroxydialkyltryptamines, on both the rat uterus and rat fundus, stimulant activity declined as the size of the alkyl group was increased although, on both tissues, the di-isopropyl compound (24) was more active than might be expected. In the 5-hydroxydibutyl compound (25) stimulant activity was very greatly reduced but, on the rat uterus, this compound had marked antagonistic activity suggesting that it had affinity but little or no efficacy.

In the dialkyltryptamines the variation of activity with structure was different from what it was with the 5-hydroxy compounds. On both preparations there was a rise up the series to the dipropyl compounds (5) followed by a fall but the results on the two preparations differed in two aspects. First, relative to tryptamine, the compounds were very much more active on the rat fundus than on the rat uterus. Second, on the rat uterus, the dimethyl compound (3)

had a very low stimulant activity. This compound was a moderate antagonist on both tissues.

The three monoalkyltryptamines(13,14, and 15) studied, appeared to resemble the tryptamines rather than 5-hydroxytryptamines in their stimulant activity. On the rat fundus however, the ethyl compound (14) was the most active and the methyl compound (13) was very feeble, but on the rat uterus, where dimethyltryptamine (3) was very feeble, methyltryptamine (13) was about as active as the ethyl (14) and propyl compounds (15). A 2-methyl group appeared to reduce both the stimulant and antagonistic activity; the stimulant properties of the 2-methyl compounds (16,17 and 18) was less than those of the simple dialkyltryptamines on both the rat uterus and rat fundus, and the antagonistic activity of 2-methyl-dimethyltryptamine (16) was less than that of dimethyltryptamine (3) on both the tissues.

On the rat uterus, the 5-benzyloxy compounds (32 to 38) were antagonists but their antagonistic activity varied with the structure in the same way as did the stimulant activity of the dialkyltryptamines (but not in the same way as did the stimulant activity of the 5-hydroxytryptamines). On the rat fundus strip, the 5-benzyloxy compounds were stimulants but the variation of activity with structure

resembled more that of the monoalkyltryptamines than that of the dialkyltryptamines and did not at all resemble that of 5-hydroxytryptamines.

Before proceeding to assess the significance of these findings, it is first necessary to make some attempt to decide the part played by amine oxidase, or any other detoxicating enzymes, and also to decide how far the receptors may be different in the different preparations.

Effects of amine oxidase.

Vane (1959) showed the existence of amine oxidase in the rat fundus strip and that marsilid potentiated the action of certain compounds which might be substrates of this enzyme. Marsilid did not change the sensitivity of the rat uterus to 5-hydroxytryptamine and tryptamine (Table 12), but when ground, this tissue contained even more amine oxidase activity than the rat fundus (Barlow, unpublished). Strips of rat fundus were incubated for 1 hr. with tryptamine (1.2 mg. base/ml. and 120 µg./ml.) and the activity of the solution compared on the rat fundus strip with that of a control kept at 4°C. for 1 hr. The destruction of tryptamine/fundus/hr. was (mean of 8 experiments ± S.E.) 52 ± 6% in the higher concentration and 93 ± 4% in the lower concentration. These figures agree quite well with

the results obtained by Vane (1959) from the oxygen uptake of ground fundus suspensions. However, there was also a destruction of 5-hydroxytryptamine when this was incubated in equimolar concentration (2.3 mg. creatinine sulphate/ml and 230 μg/ml) under similar conditions. The rates were 47 ± 4% and 74 ± 4%. Although this is significantly slower at the lower concentration than the rate of destruction of tryptamine, larger amounts of tryptamine than of 5-hydroxytryptamine are needed to cause contractions. It is possible that these results are misleading and that, in the concentrations which affect the rat fundus strip, the destruction of tryptamine by amine oxidase is proportionately greater than that of 5-hydroxytryptamine, but it is certainly not what would be expected, because the 5-hydroxytryptamine concentration in the bath is about one-thousandth of the tryptamine concentration and in the incubation experiments the enzyme appeared to be proportionately more effective with very low concentrations of substrate.

Another very puzzling finding was that, in manometric experiments, bromo-lysergic acid diethylamide in a concentration as high as 3×10^{-4} M had only slight effects (about 15% inhibition) on the oxidation of tryptamine by suspensions of ground

rat fundus. A concentration of 10^{-5} M marsilid caused 60% inhibition of this oxidation. (Vane used 3×10^{-5} M marsilid to demonstrate the potentiation of tryptamine on the rat fundus strip). It is difficult, therefore, unless Vane (1959) used unnecessarily high concentrations of marsilid, to believe that the concentrations of bromo-lysergic acid diethylamide (around 3×10^{-7} M) which antagonised 5-hydroxytryptamine more than tryptamine could also have a significant effect on the destruction of tryptamine alone. Again the difficulty arises that if the results of the incubation experiments mean anything, 5-hydroxytryptamine should be more susceptible to attack by amine oxidase than tryptamine, consequently a block of amine oxidase could not explain the results.

With such apparently contradictory results it is extremely difficult to come to any definite conclusions which are likely to be of any value. In the rat fundus strip amine oxidase does seem to play a part in the mechanism of action of tryptamine and in the rat uterus, although there is plenty of amine oxidase it does not seem to influence the actions of 5-hydroxytryptamine or tryptamine. This follows from the potentiation of tryptamine by marsilid on the rat fundus but not the rat uterus.

It would explain why α-methyltryptamines (19) and such substances as N-dipropyl-tryptamine (5) are much more active relative to tryptamine on the rat fundus than on the rat uterus. It would also explain why N-methyltryptamine (13), which is a substrate of amine oxidase (Govier, Howes and Gibbon, 1953), is only feebly active on the rat fundus strip compared with the higher homologues, which should be less susceptible to destruction by amine oxidase. Vane (1959) found that the activity of N-methyltryptamine (13) on the rat fundus strip was greatly potentiated by marsilid. On the rat uterus, the substances all had about the same activity. 5-Hydroxy-α-methyltryptamine (20) and 5-hydroxy-N-dipropyl-tryptamine (23) were not, however, more active than 5-hydroxytryptamine on either the rat uterus or the rat fundus, and, relative to 5-hydroxytryptamine, the 5-hydroxy compounds had the same order of activity on both tissues. It seems unlikely, therefore, that they are destroyed by amine oxidase to any extent. This would imply either that they do not have access to amine oxidase or that they are destroyed more rapidly by some other system. The destruction of 5-hydroxytryptamine by strips of rat fundus might be an action of a phenol oxidase not blocked by marsilid. This must obviously be investigated. The ability of a

substance to antagonise 5-hydroxytryptamine more than tryptamine on the rat fundus strip seems to depend upon different mechanisms for the destruction of 5-hydroxytryptamine and tryptamine in spite of what might be expected from the results obtained in the manometric experiments with bromo-lysergic acid diethylamide and suspensions of ground fundus. Again, these effects disappeared when the preparation is treated with marsilid, which makes it very difficult to believe that the differentiation is anything except a consequence of a block of the destruction of tryptamine.

Differences between the sensitivities of different tissues.

The relative stimulant activities of the compounds on the rat uterus and rat fundus strip were not all that dissimilar and the differences may, in part at least, be ascribed to the actions of certain of the compounds on amine oxidase in the rat fundus. Such results as have been obtained on the "D" receptors of the guinea pig ileum were similar to those obtained on the rat uterus. Effects on the "M" receptors of the guinea pig ileum and on the rabbit ear preparation seemed to be quite different from each other and from the other tissues.

There were, however, considerable differences

in the antagonistic activity of the compounds on the rat fundus and rat uterus. Bromo-lysergic acid diethylamide and 5-benzyloxygramine (41) are much less active on the rat fundus than on the rat uterus (the same dose ratios to 5-hydroxytryptamine were produced by 9×10^{-7} M 5-benzyloxygramine (41) on the rat uterus and 2×10^{-5} M 5-benzyloxygramine (41) on the rat fundus and by 10^{-8} M bromo-lysergic acid diethylamide on the rat uterus and 10^{-7} M bromo-lysergic acid diethylamide on the rat fundus). N-Dimethyltryptamine (3), 5-benzyloxy-N-dimethyltryptamine (32) and 2-methyl-dimethyltryptamine (16) however, were more active on the rat fundus than on the rat uterus. On the "D" receptors of the guinea pig ileum the activities of the antagonists studied were similar to those on the rat uterus. These results suggest that bromo-lysergic acid diethylamide and 5-benzyloxygramine (41) may act differently from N-dimethyltryptamine (3), 5-benzyloxy-N-dimethyltryptamine (32) and 2-methyl-N-dimethyltryptamine (16).

5-Hydroxytryptamine and tryptamine receptors.

The pharmacological results can be adequately explained on the assumption that 5-hydroxytryptamine

TABLE 15

Equipotent molar ratios of some tryptamines compared to 5-hydroxytryptamine on different tissues

Compound No.	DRUG	RAT FUNDUS STRIP		RAT UTERUS
		During normal tyrode	During the presence of Marsilid 3.5×10^{-5} M (Vane, 1959)	
1	5-Hydroxy-tryptamine	1.0	1.0	1.0
2	Tryptamine	933	32	210
3	N-dimethyl-tryptamine	350	110	1000
4	N-diethyl-tryptamine	112	87	500
5	N-dipropyl-tryptamine	40	41	200

and tryptamine act on the same receptors except for:-

1. The effects of increasing the concentration of bromo-lysergic acid diethylamide on the response of the rat fundus to tryptamine (p. 112).

2. The absence of any appreciable action of bromo-lysergic acid diethylamide in preventing the destruction of tryptamine by suspensions of ground rat fundus.

5. The destruction of 5-hydroxytryptamine by strips of rat fundus.

However, the relationships between structure and activity of the tryptamines resemble those of the 2-methyltryptamines but are quite different from those of the 5-hydroxy compounds. Although the activity of the tryptamines can be regarded as having two components the intrinsic activity on the receptors and the effect due to inhibition of amine oxidase, Vane's (1959) figures for the relative activity on a preparation treated with marsilid indicate (Table 15) that the peak in activity at dipropyltryptamine (5) is a real peak, not simply the result of amine oxidase block by that compound.

In these circumstances, it seems doubtful whether the compounds really are all acting on the same receptor. Although the results can be

explained using only one receptor, it may be that after all, there are 2 receptors. At the moment, there is not enough experimental evidence to justify a great deal of speculation about this.

Future work.

To obtain some idea of the central actions of these compounds it will be necessary to study them systematically on intact animals or human beings. There does not seem to be any real justification for attempting to predict their effects from the results of these *in vitro* tests. Toxicity studies are planned for 2-methyl-N-dimethyltryptamine (16) and N-dipropyltryptamine (5), which have been prepared in sufficient quantity for human trial. An investigation of the effects on the duration of sleep produced by hexobarbitone in mice was begun but was stopped because it was known that Dr. Vane was working on this. Further synthetic work should be directed towards the following:-

5-hydroxy di-*iso*-, di-*sec*, and di-*tert*- butylamines and di-amyl compounds. The actions of the compounds in antagonising the effects of nicotine and histamine on the guinea pig ileum and the effects of adrenaline on the rabbit ear should also be worth following up.

SUMMARY

Some 3-(2-dialkylaminoethyl)-, 3-(2-monoalkylaminoethyl)-, 3-(2-dialkylaminoethyl)-2-methyl, 3-(2-dialkylaminoethyl)-5-benzyloxy, 3-(2-dialkylaminoethyl)-5-hydroxy- indoles and their 3-(2-amino-n-propyl)- analogues have been studied for their ability to modify or imitate the actions of 5-hydroxytryptamine. All the compounds were tested on the isolated rat uterus and rat fundus strip preparations, some of them were also tested on the isolated guinea pig ileum, and a few were tested on the isolated perfused rabbit's ear preparation.

Most of the compounds imitated the actions of 5-hydroxytryptamine and only a few antagonised them. None was a particuarly active antagonist, the most powerful on the rat uterus being 3-(2-amino-n-propyl)-5-benzyloxy indole but this was not as active as 5-benzyloxygramine whose ability to antagonise 5-hydroxytryptamine has been known for some time. On the rat fundus strip, however, 3-(2-dimethylaminoethyl)- and 3-)2-dimethylaminoethyl)-5-benzyloxyindoles, which were the most active, were about ten times as active as 5-benzyloxygramine.

Most of the compounds caused contractions of

the rat uterus and rat fundus strip preparations. Those compounds which antagonised the contractions produced by 5-hydroxytryptamine themselves caused contractions when given in higher concentrations. The most active stimulant on the rat uterus and rat fundus strip was 3-(2-amino-n-propyl)-5-hydroxyindole, the next most potent was 3-dimethylaminoethyl)-5-hydroxyindole. These were about half as active as 5-hydroxytryptamine.

The relationships between the structure and activity of the compounds with a 5-hydroxyl group were different from those of the other compounds. The former can be regarded as true analogues of 5-hydroxytryptamine whereas the latter may be more appropriately regarded as analogues of tryptamine.

Although 5-hydroxytryptamine and tryptamine both cause contraction of the preparation used, there is some doubt whether these substances act in exactly the same way. The actions of tryptamine have been compared with those of 5-hydroxytryptamine on all the tissues. Some of the antagonists appeared to antagonise 5-hydroxytryptamine more than tryptamine and possible

explanations of this are given. The nature of the stimulant action of the new compounds has also been studied.

The possible therapeutic use of these compounds is discussed.

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